EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2): Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2):

Aliphatic primary and secondary saturated and unsaturated alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 61 flavouring substances in the Flavouring Group Evaluation 10, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the 61 substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. For four substances, information on composition of mixture and/or stereoisomerism has not been specified sufficiently.

1 On request from the Commission, Question No EFSA-Q-2011-00128, EFSA-Q-2011-00813, EFSA-Q-2011-00814 adopted on 24 March 2011.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to advise the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 61 flavouring substances in the Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 61 flavouring substances belong to chemical groups 9, 13 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The 61 flavouring substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-five of the 61 candidate substances possess one or more chiral centres and eight can exist as geometrical isomers due to the presence and the position of a double bond. For four of these substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition / composition of mixture has not been specified sufficiently.

Fifty-four of the candidate substances belong to structural class I, six of the candidate substances belong to structural class II, and one belongs to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Forty-eight of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intakes£ (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

The candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the one candidate substance assigned to structural class III has an estimated European daily *per capita* intake of 0.011 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

The combined estimated daily *per capita* intake as flavourings of the 54 candidate substances assigned to structural class I is 1600 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. Likewise, the combined estimated daily *per capita* intake as flavouring of the six candidate substances assigned to structural class II is 1.2 microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

On the basis of the data available it is concluded that there is no indication that the flavouring substances in the present Flavouring Group Evaluation possess genotoxic potential. However, the Panel reconsidered the fact that 1-hydroxypropan-2-one [FL-no: 07.169] is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person, corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore decided that further genotoxicity data are not required and that the substance could be taken through the Procedure.

It can be anticipated that, at the estimated levels of intake as flavouring substances, 58 of the alcohols, aldehydes, acetals, carboxylic acids and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products, many of which are endogenous in humans. For three [FL-no: 02.242, 06.097 and 09.824] of the flavouring substances it cannot be concluded that they are metabolised to innocuous products. Adequate margins of safety could be established for these three substances in step B4 of the Procedure.

It was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was considered that on the basis of the default MSDI approach the 61 flavouring substances, to which the Procedure have been applied, would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

The mTAMDI for the 59 flavouring substances, for which use levels information is available, range from 1600 to 5100 microgram/person/day. For 57 of these substances the mTAMDI is above the threshold of concern of their structural classes and for two substances the mTAMDI is below the threshold. The two flavouring substances which have mTAMDI intake estimates below the threshold of concern for their structural class are also expected to be metabolised to innocuous products. For two flavouring substances use levels have not been provided and no mTAMDI could be estimated. thus, for 59 flavouring substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

In order to determine whether the conclusion for the 61 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 55 flavouring substances. For two substances [FL-no: 06.135 and 08.113] information on solubility is lacking. For four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] information on composition of mixture and/or stereoisomerism has not been specified sufficiently. For one substance [FL-no: 10.063] is an identity test missing. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] pending further information.

For the remaining 57 candidate substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.088, 06.090, 06.095, 06.097, 06.102, 06.135, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874, 09.916, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.068 and 10.168] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

KEYWORDS

Flavourings, safety, lactones, saturated, unsaturated, primary, secondary, alcohols, aldehydes, acids, acetals, esters, additional oxygenated functional group, FGE.10.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

The first revision of FGE.10, FGE.10Rev1, included the assessment of eight additional candidate substances [FL-no: 06.088, 06.095, 06.102, 06.135, 09.565, 09.916, 10.040 and 10.168] and additional information on 32 substances [FL-no: 02.132, 02.198, 02.242, 06.090, 06.097, 07.169, 08.090, 09.333, 09.349, 09.360, 09.502, 09.580, 09.590, 09.601, 09.629, 09.633, 09.644, 09.683, 09.815, 09.824, 09.832, 09.862, 09.874, 10.038, 10.039, 10.043, 10.045, 10.048, 10.049, 10.052, 10.058 and 10.068] which had become available since the first FGE. Furthermore, substance [FL-no: 10.043], which can be metabolised to an alpha,beta-unsaturated ketone was withdrawn from FGE.10Rev1 to be evaluated together with other alpha,beta-unsaturated ketones in FGE.217 (EFSA, 2008b).

FGE	Opinion adopted by EFSA	Link	No. Of candidate substances
FGE.10	28 October 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1232_en.html	51
FGE.10Rev1	30 January 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902296182.htm	58
FGE.10Rev2	23 March 2011		61

The present revision of FGE.10, FGE.10Rev2, includes the assessment of three additional candidate substances [FL-no: 08.113, 10.059 and 10.063]. No toxicity or metabolism data were provided for these three substances.

A search in open literature revealed data on metabolism, genotoxicity, repeated dose toxicity as well as reproductive/developmental toxicity for [FL-no: 08.113] but not for [FL-no: 10.059 and 10.063].

FGE.10Rev2 also include additional information submitted by industry on the stereoisomeric composition/composition of mixture requested in FGE.10Rev1 for eight substances [FL-no: 06.088, 06.095, 06.135, 09.565, 09.916, 10.038, 10.040 and 10.168], as well as identity information for [FL-no: 06.088 and 06.095].

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register prior to their authorisation and inclusion in a Union List according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 10, Revision 1

1.1. Description

The present Flavouring Group Evaluation 10, Revision 2 (FGE.10Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 61 alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones from chemical groups 9, 13 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 61 flavouring substances (candidate substances) under consideration are listed in Table 1, as well as their chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications.

The outcome of the Safety Evaluation is summarised in Table 2a.

Fourteen candidate substances are aliphatic lactones [FL-no: 10.038, 10.039, 10.040, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068 and 10.168]; thirty-one candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874 and 09.916]; six candidate substances are acetals [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135]; one candidate substance is an alpha-hydroxyacid [FL-no: 08.090]; one candidate substance is a ketoalcohol [FL-no: 07.169]; one candidate substance is an alkoxy-alcohol [FL-no: 02.242]; two candidate substances are diols [FL-no: 02.132 and 02.198]; one candidate substance is a dialdehyde [FL-no: 05.149] and four candidate substances are aliphatic dicarboxylic acids [FL-no: 08.053, 08.082, 08.103 and 08.113].

The 61 candidate substances are structurally related to 29 aliphatic lactones (supporting substances) evaluated at the 49th JECFA meeting (JECFA, 1998a) and to 47 aliphatic primary alcohols, aldehydes, carboxylic acids, acetals and esters containing additional oxygenated functional groups evaluated at the 53rd JECFA meeting (JECFA, 2000c). These supporting substances are listed in Table 3, together with their evaluation status.

The hydrolysis products of candidate esters and acetals as well as their evaluation status are listed in Table 2b.

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different, they may have different chemical properties resulting in possible variation of their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Thirty-five of the substances possess one or more chiral centres [FL-no: 02.132, 02.198, 06.088, 06.090, 06.095, 06.135, 08.090, 09.333, 09.346, 09.349, 09.360, 09.502, 09.580, 09.590, 09.601, 09.629, 09.633, 09.644, 09.683, 09.815, 09.824, 09.832, 09.862, 09.874, 09.916, 10.038, 10.039, 10.040, 10.045, 10.048, 10.049, 10.052, 10.058, 10.068 and 10.168]. In all cases the stereoisomeric composition has been specified.

Due to the presence and the position of a double bond, eight of the 61 substances can exist as geometrical isomers [FL-no: 09.350, 09.351, 09.565, 10.038, 10.039, 10.040, 10.059 and 10.063]. For four of the substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition / composition of mixture has not been specified sufficiently. Industry has informed that [FL-no: 10.038 and 10.040] exists as a mixture of (Z)- and (E)-isomers (EFFA, 2010a), however, the composition of the isomeric mixture has to be provided.

1.3. Natural Occurrence in Food

Forty-eight of the 61 flavouring substances have been reported to occur in one or more of the following food items: fruits (apple, pineapple, melon, guava, banana, starfruit, papaya, raspberry, mango, plum), juice, butter, meat, cheese, skimmed milk powder, green tea, coffee, beer, wine and whisky.

Quantitative data on the natural occurrence in food have been reported for thirty-eight of the candidate substances. These reports include:

- Octane-1,3-diol [FL-no: 02.198]: up to 21 mg/kg in apple and up to 95.1 mg/kg in apple juice.
- Hexadecano-1,5-lactone [FL-no: 10.049]: up to 10.6 mg/kg in butter and up to 1.3 mg/kg in heated lamb and mutton fat.
- Hexadecano-1,4-lactone [FL-no: 10.048]: up to 16.7 mg/kg in heated butter.
- 2-Butoxyethan-1-ol [FL-no: 02.242]: 0.02 mg/kg in mozzarella cheese.
- Hexadecano-1,16-lactone [FL-no: 10.047]: 0.0145 mg/kg in skimmed milk powder.
- Heptano-1,5-lactone [FL-no: 10.045]: up to 0.4 mg/kg in green tea.
- 1-Hydroxypropan-2-one [FL-no: 07.169]: up to 4 mg/kg in coffee.
- 2-Ethyl-4-methyl-1,3-dioxolane [FL-no: 06.088]: up to 2 mg/kg in port wine.
- 4-methyl-2-propyl-1,3-dioxolane [FL-no: 06.095]: up to 2 mg/kg in port wine.
- 1,1,3-Triethoxypropane [FL-no: 06.097]: up to 3 mg/kg in pear brandy and less than 0.8 mg/kg in whisky.

- 2-Isobutyl-4-methyl-1,3-dioxolane [FL-no: 06.135]: up to 2 mg/kg in port wine.
- Nonanedioic acid [FL-no: 08.103]: up to1.5 mg/kg in beer.
- Propyl lactate [FL-no: 09.815]: trace amount in white wine.
- Isobutyl lactate [FL-no: 09.590]: 20 mg/kg in port wine.
- Ethyl 3-hydroxyoctanoate [FL-no: 09.916]: up to 0.05 mg/kg in papaya, 0.02 mg/kg in orange juice and 0.03 mg/kg in grapefruit juice.

According to TNO, 13 of the substances have not been reported in any food items. These substances are listed in Table 1.1 (TNO, 2000; TNO, 2010).

1.1 Candidate substances not reported to occur in nature (TNO, 2000; TNO, 2010)

FL-no	Name
06.102	2-hexyl-5-hydroxy-1,3-dioxane
08.113	Succinic acid, disodium salt
09.502	ethyl butyryl lactate
09.633	methyl 5-hydroxydecanoate
09.644	methyl lactate
09.824	ethyl 2-acetylbutyrate
09.832	ethyl 3-acetohexanoate
09.833	iso-propyl 4-oxopentanoate
09.874	di(2-methylbutyl) malate
10.040	dec-8-eno-1,5-lactone
10.059	hexadec-7-en-1,16-lactone
10.063	hexadec-9-en-1,16 lactone
10.068	pentadecano-1,14-lactone

2. Specifications

Purity criteria for the 61 substances have been provided by the Flavouring Industry (EFFA, 2003c; EFFA, 2004ag; Flavour Industry, 2011a) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), this information is adequate for 58 of the 61 substances. For one substance [FL-no: 10.063] an identity test is missing and for two substances [FL-no: 06.135 and 08.113] are information on solubility in water and ethanol lacking.

Furthermore, information on geometrical stereoisomerism is needed for four of the candidate substances (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) (SCF, 1999) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

The total annual volumes of production of the 61 candidate substances from use as flavouring substances in Europe has been reported to be approximately 13160kg (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b). For the 76 supporting substances the annual volume of production is 357000 kg (JECFA, 1999b; JECFA, 2000b).

On the basis of the annual volumes of production reported for the 61 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a).

98 % of the total annual volume of production for the candidate substances is accounted for by three of these flavouring substances, succinic acid disodium salt [FL-no: 08.113], hexadec-9-en-1,16-lactone [FL-no: 10.063] and diethyl maleate [FL-no: 09.351]. The estimated daily *per capita* intake of succinic acid disodium salt from use as a flavouring substance is 1500 microgram, that of hexadec-9-

[4] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

en-1,16-lactone is 48 microgram and that of diethyl maleate is 12 microgram. The daily *per capita* intakes for each of the remaining substances are less than 10 microgram (Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For 59 of the 61 candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a);. For two substances [FL-no: 06.135 and 08.113] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000.

The 59 candidate substances, for which use levels have been provided, are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the 59 candidate substances, for which use levels have been provided, are in the range of 1 - 20 mg/kg food, and the maximum use levels are in the range of 5 - 100 mg/kg (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

The mTAMDI values for the 53 candidate substances from structural class I, for which use levels have been reported, range from 1600 to 5100 microgram/person/day, for the five candidate substances from structural class II, for which use levels are available, the mTAMDI range from 3800 to 3900 microgram/person/day for each. For the candidate substance from structural class III the mTAMDI is 4100 microgram/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

[5] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[7] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All*
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All *
03.0	Edible ices, including sherbet and sorbet	All*
04.1	Processed fruits	All*
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
05.0	Confectionery	All*
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All*
07.0	Bakery wares	All*
08.0	Meat and meat products, including poultry and game	All*
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All* except [FL-no: 08.090]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All* except [FL-no: 06.095, 09.644]
13.0	Foodstuffs intended for particular nutritional uses	All* except [FL-no: 06.095, 09.644]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All*
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All*
15.0	Ready-to-eat savouries	All*
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All*

* Information on use levels has not been provided for [FL-no: 06.135 and 08.113]

4. Absorption, Distribution, Metabolism and Elimination

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic and neutral media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured. Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclication are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones with low molecular weight being uncharged may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte. The hydroxycarboxylic acid obtained from lactone hydrolysis enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by 2-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments are then metabolised in the citric acid cycle. The Panel anticipated that the two unsaturated omega-lactones (10.059, hexadec-7-en-1,16-lactone and 10.063, hexadec-9-en-1,16-lactone) are metabolised like the structurally related saturated lactones, namely through ring opening followed by fatty acid degradation.

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma-, delta- and omega-lactones, lactones fused to alicyclic rings such as 2-(2-hydroxycyclopent-4-enyl)ethanoic acid gamma-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for

by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993).

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto alcohols) and acid components (i.e. alpha-, beta- or gamma-keto or hydroxy acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised and excreted. It has to be noted that the 2-acetyl butyric acid, formed as one of the hydrolysis products of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], has some structural similarities to valproic acid, which, together with a number of its derivatives, has been recognised as teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Although it can be predicted that 2-acetylbutyric acid is further metabolised through the usual pathways of detoxication for carboxylic acids (i.e. mainly *via* glucuronidation reaction), the structural similarity with valproic acid does not allow the prediction that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised only to innocous products.

The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters. The most probable metabolic reactions of the hydrolysis products are, oxidation of alcohols to aldehydes and acids, conjugation of alcohols and acids to glucuronides and sulphates and beta- and omega-oxidation of carboxylic acids.

Beta-keto acids and derivatives like acetoacetic acid undergo ready decarboxylation. Along with alpha-keto and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways. The gamma-keto acids and related substances may undergo complete or partial beta-oxidation to yield metabolites that are eliminated in the urine. Omega-substituted derivatives are readily oxidised and/or excreted in the urine. Simple aliphatic di- and tricarboxylic acids participate in the tricarboxylic acid cycle. For instance, succinic acid is a normal intermediary metabolite and a constituent of the citric acid cycle; it occurs normally in human urine (1.9-8.8 mg/L). Succinic acid is readily metabolized when administered to animals, but may be partly excreted unchanged in the urine if large doses are given (Patty, 1993), Vol. II, p. 3579).

One of the candidate substances, 1-hydroxypropan-2-one [FL-no: 07.169] (acetol), is a metabolite of acetone, which is an endogenous substance formed from the degradation of body fat / fatty acids. The major metabolic pathway in mammals of acetone at low blood concentrations (i.e. in healthy humans not exposed to external sources, acetone occurs in amounts of approximately 4 - 12 mg per person, corresponding to approximately 0.7 to 2 mg/l blood (Dick el al., 1988; Ashley et al., 1994; Wang et al, 1994c), is via the methylglyoxal route, where acetone is first oxidised to 1-hydroxypropan-2-one, which is then oxidised to 2-oxopropanal (methylglyoxal [FL-no: 07.001]). 2-Oxopropanal will after further metabolism give rise to glucose (Morgott, 1993; WHO, 1998a; NAS/COT, 2005).

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may be expected to undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption. Once hydrolysed, the component alcohols and aldehydes are expected to be metabolised primarily through the above mentioned common routes of biotransformations and excreted.

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation. It may be anticipated that glutaraldehyde [FL-no: 05.149] is biotransformed through the common pathways of detoxication of aldehydes to innocuous products.

Among the candidate substances, an alkoxy-alcohol, 2-butoxyethanol [FL-no: 02.242], is mainly metabolised to butoxyacetic acid, which has been identified as the metabolite responsible for the haemolysis of red blood cells induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with a second oxygenated functional group and aliphatic lactones included in the present FGE are generally metabolised to innocuous products (many of which are endogenous in humans), at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethan-1-ol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound.

A more detailed description of the metabolism of the candidate substances in this FGE is given in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], should not be evaluated through the Procedure until new data became available because it was found to be genotoxic *in vitro* in bacterial assays. However, in the first revision of FGE.10 (FGE.10Rev1) the Panel reconsidered this compound and concluded that it is an endogenous metabolite of acetone which is formed from the degradation of body fat/fatty acids and that it would be further metabolised to innocuous compounds, and thus not be of concern at the exposure levels resulting from its use as a flavouring substance (see Section 8.4, conclusion on the genotoxicity). The Panel therefore decided that 1-hydroxypropan-2-one [FL-no: 07.169] could be evaluated along the A side of the Procedure in FGE.10Rev1.

For the safety evaluation of the 61 candidate substances in the present revision of FGE.10 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 61 substances are summarised in Table 2a.

Step 1

Fifty-four of the candidate substances are classified according to the decision tree approach by Cramer *et al.* (1978) into structural class I, six are classified into structural class II [FL-no: 02.242, 06.088, 06.090, 06.095, 06.097 and 06.135], and one into structural class III [FL-no: 06.102].

Step 2

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol; 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance with structural similarities to 2-butoxyethanol; and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Therefore, these substances are evaluated via the B-side of the Procedure. The evaluation of the remaining 58 candidate substances proceeds via the A-side of the Procedure.

Step A3

Step A3 applies to 53 candidate substances from structural class I [FL-no: 02.132, 02.198, 05.149, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.832, 09.833, 09.862, 09.874, 09.916, 10.038, 10.039, 10.040, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068 and 10.168], four candidate substances from structural class II [FL-no: 06.088, 06.090, 06.095 and 06.135] and one candidate substance from structural class III [FL-no: 06.102].

The 53 candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The four candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the one candidate substance assigned to structural class III has an estimated European daily *per capita* intake of 0.011 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

Accordingly, these 58 candidate substances do not pose a safety concern when used at estimated levels of intake as flavouring substances, based on the MSDI approach.

Step B3

The MSDIs of the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242], 1,1,3-triethoxypropane [FL-no: 06.097] and ethyl 2-acetylbutyrate [FL-no: 09.824], were estimated to be 0.0012 microgram/*capita*/day for each. Thus, the MSDI-values of all three candidate substances are below the threshold of concern for their structural classes of 540 microgram/person/day (class II) for [FL-no: 02.242 and 06.097] and of 1800 microgram/person/day (class I) for [FL-no: 09.824]. Accordingly, the three substances proceed to step B4 of the Procedure.

Step B4

The candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824] is expected to be hydrolysed to the corresponding alpha-ethylated carboxylic acid, 2-acetylbutyric acid and ethanol. No toxicity studies that would permit establishing a No Observed Adverse Effect Level (NOAEL) are available for ethyl 2-acetylbutyrate or its hydrolysis product 2-acetylbutyric acid. 2-Acetylbutyric acid is structurally related to 2-ethylhexanol [FL-no: 02.082] for which the JECFA has established an ADI of 0.5 mg/kg bw/day (JECFA, 1993b). The estimated daily *per capita* intake, based on the MSDI approach and expressed in microgram/kg bw/day for the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate (and 2-acetylbutyric acid) is approximately 25 x 10^6 fold below the acceptable daily intake (ADI) value of the structurally related 2-ethylhexanol. Furthermore, the hydrolysis product, 2-acetylbutyric acid, shows some structural similarities to valproic acid, a known teratogenic compound. If 2-acetylbutyric acid is considered to be as potent as valproic acid (NOAEL = 600 mg/day) the margin of safety would be 5 x 10^8, based on the MSDI of 0.0012 microgram/*capita*/day. Accordingly,

it is concluded that ethyl 2-acetylbutyrate [FL-no: 09.824] does not pose a safety concern at the estimated level of intake, based on the MSDI approach.

For the candidate substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-triethoxypropane [FL no: 06.097], the hydrolysis product of which has structural similarities to 2-butoxyethan-1-ol, a NOAEL could not be established in sub-chronic/chronic toxicity studies with respect to haemotoxicity. Thus, strictly according to the Procedure additional toxicity data would be needed to finalise the evaluation of these two substances in step B4 of the Procedure. However, reconsidering and updating the previous version of this FGE, the Panel noted that at least for 2-butoxyethan-1-ol [FL-no: 02.242] a wealth of toxicity data is available, so that this substance can be evaluated on a broader basis than only the Procedure for the Evaluation of Flavouring substances, which in principle has been designed for the evaluation of data-poor substances.

Considering the data available, especially those on kinetics and mechanism of action (see US-EPA, 1999 and draft EU-RAR 2007, human health part) it becomes clear that there are major differences in sensitivity between humans and rats regarding the prime toxic effect (haemotoxicity) of this substance, with humans (together with dog, guinea pig, pig, cat and rabbit) being considerably less sensitive than rats (together with mouse, hamster and baboon). For that reason it seems inappropriate to ask for further toxicity data in animals, as the available data already cover the most sensitive species. In this case an alternative approach is needed and possible for this data-rich substance (EPA, 1999; EU-RAR, 2007).

In their evaluation, US-EPA, using a Bench Mark Dose approach, combined with physiologically-based kinetic modelling arrived at an oral Reference dose (RfD) for chronic exposure of 0.5 mg/kg body weight (bw)/day (EPA, 1999).

In the EU-RAR (2007) a Human equivalent Lowest Observed Adverse Effect Level (LOAEL) of 9.5 mg/kg bw/day is used, which was derived from the LOAEL in the rat using the same kinetic models as applied by US-EPA. A Margin of Safety of 3 between the Human equivalent LOAEL and estimates for chronic exposure of "Consumers" or "Humans, exposed via the Environment" was considered sufficient to reach a conclusion of no concern.

For each of the two candidate flavouring substances 2-butoxyethan-1-ol [FL-no: 02.242] and 1,1,3-tri-ethoxypropane [FL no: 06.097] an MSDI of 0.0012 microgram/*capita*/day (see Table 6.1) can be calculated. The Reference dose (RfD) from US-EPA and the LOAEL from the draft EU-RAR are factors of 2.5×10^7 or 4.75×10^8 above the MSDI, respectively. The Panel concluded that these margins are sufficiently large to decide that based on the MSDI exposure estimates, these substances are of no concern when used as flavouring substances.

In conclusion the Panel considered that all 61 candidate substances evaluated through the Procedure were of no safety concern at the estimated levels of intake based on the MSDI approach.

6. Comparison of the Intake Estimations Based on the MSDI Approach and the mTAMDI Approach

The mTAMDI for the 53 candidate substances in structural class I and for which use levels information is available, range from 1600 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day.

The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day.

For the one substance from structural class III the mTAMDI is 4100 microgram/person/day, which is above the threshold of 90 microgram/person/day.

Thus for the 57 candidate substances further information is required as the mTAMDIs are above the threshold for the structural class. This would include more reliable intake data and then, if required, additional toxicological data. For two substances [FL-no: 06.135 and 08.113] use levels are required for the food categories as listen in Commission Regulation (EC) No 1565/2000 (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

For comparison of the MSDI- and mTAMDI-values see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	0.0061	3900	Class I	1800
02.198	Octane-1,3-diol	0.0012	3900	Class I	1800
05.149	Glutaraldehyde	0.055	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	0.22	1600	Class I	1800
08.053	Malonic acid	0.0012	3200	Class I	1800
08.082	Glutaric acid	0.0012	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	0.0012	3800	Class I	1800
08.103	Nonanedioic acid	0.0012	3200	Class I	1800
08.113	Succinic acid, disodium salt	1500		Class I	1800
09.333	sec-Butyl lactate	3.7	3900	Class I	1800
09.345	Di-isopentyl succinate	0.037	3900	Class I	1800
09.346	Dibutyl malate	0.0012	3900	Class I	1800
09.347	Dibutyl succinate	0.12	3900	Class I	1800
09.348	Diethyl adipate	0.027	3900	Class I	1800
09.349	Diethyl citrate	0.12	3900	Class I	1800
09.350	Diethyl fumarate	0.0012	3900	Class I	1800
09.351	Diethyl maleate	12	3900	Class I	1800
09.352	Diethyl nonanedioate	0.0012	3900	Class I	1800
09.353	Diethyl oxalate	0.0012	3900	Class I	1800
09.354	Diethyl pentanedioate	0.0012	3900	Class I	1800
09.360	Ethyl 2-acetoxypropionate	4.9	3900	Class I	1800
09.502	Ethyl butyryl lactate	0.5	3900	Class I	1800
09.558	Dimethyl malonate	0.097	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	0.74	3900	Class I	1800
09.580	Hexyl lactate	0.49	3900	Class I	1800
09.590	Isobutyl lactate	3.7	3900	Class I	1800
09.601	Isopentyl lactate	7.2	5100	Class I	1800
09.626	Methyl 2-oxopropionate	0.024	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	0.0012	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	0.24	3900	Class I	1800
09.634	Methyl acetoacetate	0.012	3900	Class I	1800
09.644	Methyl lactate	0.34	3600	Class I	1800
09.683	Pentyl lactate	0.61	3900	Class I	1800
09.815	Propyl lactate	0.62	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	0.33	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	0.24	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	0.0012	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	0.015	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	0.011	3900	Class I	1800
10.038	Dec-7-eno-1,4-lactone	0.37	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	1.2	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	0.011	3900	Class I	1800
10.045	Heptano-1,5-lactone	0.012	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	0.024	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	0.0061	3900	Class I	1800
10.049	Hexadecano-1,5-lactone	0.024	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	0.61	3900	Class I	1800
10.055	Pentano-1,5-lactone	0.012	3900	Class I	1800
10.058	Tridecano-1,5-lactone	0.61	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	1.9	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	48	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	0.9	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	1.2	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	0.0012	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	0.0061	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	0.012	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	0.012	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane	1.2		Class II	540

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.242	2-Butoxyethan-1-ol	0.0012	3900	Class II	540
06.097	1,1,3-Triethoxypropane	0.0012	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	0.011	4100	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2000c; EFFA, 2003d; EFFA, 2008b), the combined estimated daily *per capita* intake as flavourings of the 54 candidate flavouring substances assigned to structural class I is 1600 microgram, of the six candidate flavouring substances assigned to structural class II is 1.2 microgram and of the one candidate substance assigned to structural class III, 0.01 microgram. These estimates do not exceed the thresholds of concern for the correspondig structural classes of 1800, 540 and 90 microgram/person/day, respectively.

The 14 candidate lactones are structurally related to 27[8] supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about 55 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 40 candidate substances are structurally related to 32[9] supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 25000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 24000 microgram/*capita*/day.

[8] European production volumes are only available for 27 of the 29 JECFA evaluated lactones – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.

[9] European production volumes are only available for 32 of the 47 JECFA evaluated alcohols and related substances – these substances have been evaluated by JECFA before 2000 and accordingly no EFSA considerations have been performed including requests for production volumes.

8. Toxicity

8.1. Acute Toxicity

Data are available for 15 of the candidate substances (Annex IV, Table IV.1). For the majority of candidate substances, oral LD_{50} values, in mice or rats, varied from 100 mg/kg up to more than 5000 mg/kg bw. For butane-1,3-diol [FL-no: 02.132] and octane-1,3-diol [FL-no: 02.198] LD_{50} values between 20 g/kg bw/day and approximately 30 g/kg bw/day are reported (Annex IV, Table IV.1).

Forty-nine supporting substances were tested for acute toxicity in mice and/or rats (Annex IV, Table IV.1). For the majority of the supporting substances, oral LD_{50} values, in mice or rats, varied from 1300 mg/kg up to 18500 mg/kg bw. For diethyl sebacate [FL-no: 09.475] and tributyl acetylcitrate [FL-no: 09.511] LD_{50} values larger than 30 g/kg body weight (bw) are reported.

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute/subchronic/chronic toxicity data are available for five candidate substances, 2-butoxyethanol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], glutaraldehyde [FL-no: 05.149], nonanedioic acid [FL-no: 08.103] and for 25 supporting substances of the present Flavouring Group Evaluation (JECFA, 1998a; JECFA, 2000c) (Annex IV, Table IV.2).

Available data on repeated dose toxicity show that haemolysis is the primary and critical response elicited in the main animal test models (rats and mice) following oral exposure to 2-butoxyethan-1-ol, in which the haematotoxic action is produced by the metabolite butoxyacetic acid (this effect is also seen following other exposure routes such as inhalation or dermal exposure. These exposure routes are not considered relevant for this evaluation as data from oral exposure are available. Notably, the haematotoxic effect exhibits a pronounced species difference. In sensitive species (rat, mouse, hamster, baboon), 2-butoxyethan-1-ol produces a characteristic toxicity that is revealed clinically by the appearance of haemoglobinuria and pathologically by changes in a variety of blood parameters (EPA, 1999; EU-RAR, 2004a). LOAELs of 69 and 82 mg/kg bw/day (slight decrease in body weight gain, haematological and liver effects) have been reported for male and female rats, respectively (NTP, 1993a). Human erythrocytes are about 100-times less sensitive than rat erythrocytes as judged by prehaemolytic changes *in vitro* (increase in mean erythrocyte volume, erythrocyte deformability) consistently observed in both species. Studies have also shown that potentially sensitive human sub-populations, including children, the elderly and those with sickle cell anemia, do not show increased sensitivity to the haemolytic action of 2-butoxyethan-1-ol. Furthermore, the *in vivo* blood concentrations producing haemolysis in the animal experiments are considered unlikely to occur under normal conditions of human exposure to 2-butoxyethan-1-ol (EU-RAR, 2004a).

Carcinogenicity: In a two year inhalation study, F344/N rats were exposed to 0, 0.031, 0.0625 and 0.125 mg/m^3 and $B6C3F_1$ mice were exposed to 0, 0.0625, 0.125 and 0.250 mg/m^3 2-butoxyethan-1-ol; (NTP, 1993a). The exposure caused a low incidence of haemangiosarcoma in male mice at the highest exposure concentration; haemangiosarcoma did not occur in female mice or in rats. In female mice, 2-butoxyethan-1-ol caused an increased incidence of forestomach tumours. It was not carcinogenic in rats. The occurrence of haemangiosarcoma in male mice only at highest exposure concentration is suggestive of a threshold phenomenon, related to the induction of haemolysis in rodent species. With regard to human relevance, the mechanism proposed for the induction of haemangiosarcomas strongly supports the conclusion that 2-butoxyethan-1-ol is unlikely to be a carcinogenic hazard at the estimated level of intake as flavouring substance, because human erythrocytes are demonstrably more resistant to haemolysis than are rodent erythrocytes.

Glutaraldehyde[10] [FL-no: 05.149] (50, 250, 1000 mg/l in drinking water, resulting in doses of 2.9-6.9, 14.5-31.8 and 54.7-104.6 mg/kg/day, respectively) was not tumorigenic in a two year carcinogenicity study on male and female rats (Van Miller et al., 2002). Furthermore, malonic acid [FL-no: 08.053] was negative in a liver foci tumour promotion assay.

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data on developmental toxicity and reproductive toxicity are available for the following five candidate substances: 2-butoxyethan-1-ol [FL-no: 02.242], butane-1,3-diol [FL-no: 02.132], glutaric acid [FL-no: 08.082], glutaraldehyde [FL-no: 05.149] and nonanedioic acid [FL-no: 08.103]. Studies for supporting substances comprise: Butyro-1,4-lactone [FL-no: 10.006] and adipic acid [FL-no: 08.026] (JECFA, 1998a; JECFA, 2000c) (Annex IV, Table IV.3).

For 2-butoxyethan-1-ol [FL-no: 02.242] no effects on fertility were observed in female and male mice given 2-butoxyethan-1-ol in the drinking water in a continuous breeding study in which a NOAEL of 720 mg/kg was derived (EU-RAR, 2004a). As to developmental toxicity, studies performed on animals via various administration routes did not demonstrate any teratogenic potential, and foetotoxicity and embryotoxicity (lethality and resorptions) were only observed in the presence of maternal toxicity (regenerative haemolytic anaemia). Other effects seen on foetuses were an increase in the incidence of skeletal variations, which are generally described as ossification delays. The effects seen in developmental toxicity studies with 2-butoxyethan-1-ol are considered to result from haemolysis and subsequent maternal anemia (EU-RAR, 2004a). Overall, 2-butoxyethan-1-ol is not considered to pose a safety concern with respect to reproduction and development at the estimated level of intake as flavouring substance.

No information is available on ethyl 2-acetyl butyrate [FL-no: 09.824], the hydrolysis product of which, 2-acetyl butyric acid, has some structural similarities to valproic acid, which, together with a number of its derivatives, has been recognised as teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Offspring of mothers using > 1000 mg valproic acid per day were at a significantly increased risk of major congenital malformations especially neural tube defects, compared to offspring exposed < or 600 mg valproic acid/day (RR 6.8; 95 % CI: 1.4-32.7). No difference in risk of major congenital malformations was found between the offspring exposed to 601 – 1000 mg/day and < or = 600 mg/day. Thus, 600 mg/day is considered as NOAEL for the teratogenic effects of valproic acid in humans.

Developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

[10] Glutaraldehyde is also used in food contact material (FCM). It was evaluated by the former Scientific Committee on Food (SCF List 7, http://europa.eu.int/comm/food/fs/sc/scf/out50_en.pdf), however, this is not a final evaluation. According to German recommendations, glutardialdehyde (synonym: glutaraldehyde) may be used for the production of artificial sausage skin (maximum use level 0.1 %). The maximum residual amount of glutardialdehyde is 50 mg per kg artificial sausage skin (ready for use). Furthermore, glutardialdehyde may be used as anti slime agent for the production of paper as FCM (maximum use level 2.5 % based on dry fibre material). The maximum residual amount of glutardialdehyde is 2 mg per kg paper (ready for use). The Panel noted that maximum residual amounts of glutaraldehyde in food contact material (as set e.g. in German recommendations) could apparently conflict with reported use levels of glutaraldehyde as flavouring. However, in the German recommendations, the maximum residual amounts were set considering the technologically needed use levels (limited data submitted) rather than on toxicological data, and the Panel therefore did not find the low maximum residual amounts for glutaraldehyde as such in conflict with higher use levels for glutaraldehyde as flavouring, which could therefore go through the Procedure.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 11 of the candidate substances. These 11 substances are pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168], glutaraldehyde [FL-no: 05.149], 1-hydroxypropan-2-one [FL-no: 07.169], butane-1,3-diol [FL-no: 02.132], malonic acid [FL-no: 08.053], diethyl maleate [FL-no: 09.351] diethyl adipate [FL-no: 09.348], methyl acetoacetate [FL-no: 09.634], 2-butoxyethan-1-ol [FL-no: 02.242] and glutaric acid [FL-no: 08.082]. There were genotoxicity data on 26 supporting substances (Annex IV, Table IV.4 and IV.5).

In vitro

Pentano-1,5-lactone [FL-no: 10.055], 5,6-dimethyl-tetrahydro-pyran-2-one [FL-no: 10.168] and methyl acetoacetate [FL-no: 09.634] were reported to be negative in microbial mutagenicity assays.

1-Hydroxypropan-2-one [FL-no: 07.169] was positive in Ames tests using strains TA100 and TA104 in the presence and absence of S-9 metabolic activation (Garst et al., 1983; Marnett et al., 1985a; Yamaguchi, 1982; Yamaguchi and Nakagawa, 1983). These results are consistent across the four reported studies which, despite limitations in study design and reporting, suggest that 1-hydroxypropan-2-one should be considered an *in vitro* mutagen in bacteria. There are no data provided on either *in vitro* endpoints nor on *in vivo* studies.

Diethyl maleate [FL-no: 09.351] was reported to produce mutations in the TK +/- locus of L5178Y mouse lymphoma cells. However, the concentration required for a two-fold increase of mutations results in 70 % growth reduction (Wangenheim and Bolcsfoldi, 1988), rendering this effect questionable. Diethyl maleate was positive in an aneuploidy test using V79 Chinese hamster lung cells at 8.7 $x10^{-6}$ M but not at 5.2 $x10^{-6}$ M (Önfelt, 1987); generally aneuploidy is considered as a threshold phenomenon.

In vitro and/or in vivo

Glutaric acid [FL-no: 08.082] was reported to be negative in the Ames and Rec test as well as in an *in vivo* test for rat bone marrow aberrations.

2-Butoxyethan-1-ol [FL no: 02.242] was negative in the Ames test and in *in vitro* tests in mammalian cells for induction of forward mutations, chromosomal aberrations and sister chromatid exchanges (SCE). Positive results were only reported in one study in V79 cells (for induction of forward mutations, SCE and micronuclei) at doses above the maximum level recommended by current OECD Guidelines. Equivocal positive results were reported in an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes. *In vivo*, negative results were obtained in an adequate micronucleus tests in rats and mice following oral or intraperitoneal administration. No evidence of DNA binding or alteration of DNA methylation was obtained in a study in rats and mice. The overall experimental evidence indicated that 2-butoxyethan-1-ol is not genotoxic (see Table IV.5).

Glutaraldehyde [FL-no: 05.149] exhibits genotoxic effects in *in vitro* tests, most consistently in the bacterial mutagenicity assays. Forward gene mutation tests *in vitro* in mammalian cells have given variable results depending on the locus: negative with HGPRT and positive with TK. Also, SCE, chromosome aberration and UDS tests have shown no effect to a weakly positive effect, depending on the laboratory, protocol, dosages and sampling times. However, that any *in vitro* potential for genotoxic effects will not be expressed *in vivo* is indicated by the *in vivo* study results, which include chromosomal aberrations, mammalian erythrocyte micronucleus test, UDS and recessive lethal mutations. The only study suggesting an *in vivo* effect was an increase in micronuclei in mouse blood cells up to 15 mg/kg bw. However, the data are insufficiently reported. The negative results from the well-conducted *in vivo* studies may be related to the rapid metabolism and protein binding characteristics of glutaraldehyde, and the related observation that although 14C-labelled glutaraldehyde may be detected in cell cytoplasm there is no nuclear fraction radioactivity (Vergnes and Ballantyne, 2002).

Butane-1,3-diol [FL-no: 02.132] was reported as not inducing chromosomal aberration in bone marrow and was negative in a rat dominant lethal assay. Butane-1,3-diol [FL-no: 02.132] was checked for cytogenetic effects over a period of three generations at doses of 5 % (5000 mg/kg/day), 10 % and 24 %. None of the doses produced abnormal rates of bone marrow metaphase cells as compared to controls (Hess et al., 1981).

Malonic acid [FL-no: 08.053] was found negative in a rat liver foci assay, diethyl adipate [FL-no: 09.348] was reported to be negative in a mouse dominant lethal assay.

Genotoxicity tests are available for 28 supporting substances. Some positive test results from *in vitro* studies are reported for 4-hydroxybutyric acid lactone [FL-no: 10.006], which, however, was found negative in a reliable *Drosophila in vivo* sex-linked recessive lethal mutation assay (Table IV 4 and 5). Results of *in vivo* bone marrow micronucleus assays in mice available for 4-hydroxybutyric acid lactone were also negative, however, since the PCE/NCE ratio was not reported it is not clear if the test substance reached the bone marrow (Table IV.5). Positive *in vitro* data that cannot be evaluated are reported for hexano-1,5-lactone [FL-no: 10.010], nonano-1,4-lactone [FL-no: 10.001], undecano-1,4-lactone [FL-no: 10.002], undecano-1,5-lactone [FL-no: 10.011] and ethyl acetoacetate [FL-no: 09.402] (Annex IV, Table IV.4).

Conclusions on genotoxicity

Genotoxicity data are only available on a very limited number of the candidate substances in this Flavouring Group Evaluation and none has a complete package of mutagenicity endpoints.

One of the candidate substances (1-hydroxypropan-2-one) induced gene mutations in bacteria but has not been studied *in vivo* or in other *in vitro* assays.

In its first evaluation of this group of aliphatic alcohols, aldehydes, acetals, carboxylic acids and esters containing an additional oxygenated functional group and lactones (EFSA, 2005b) the Panel considered that for the candidate substance, 1-hydroxypropan-2-one [FL-no: 07.169], it was necessary to request additional *in vitro* data from studies in mammalian cells. However, in this revision of FGE.10 FGE.10Rev1) the Panel reconsidered the fact that 1-hydroxypropan-2-one is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4-12 mg/person corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore concluded that 1-hydroxypropan-2-one [FL-no: 07.169] would not be of safety concern at the exposure level resulting from its use as a flavouring substance. Consequently, the Panel decided that further studies on the *in vitro* genotoxicity of 1-hydroxypropan-2-one [FL-no: 07.169] would not be required.

Glutaraldehyde was tested *in vitro* and *in vivo*, with positive findings *in vitro*. However, based upon the negative results of *in vivo* genotoxicity assays, along with the lack of tumorigenicity in mice and rats, the *in vitro* genotoxicity data are not considered relevant for the safety evaluation of glutaraldehyde.

Disodium succinate did not induce mutations in bacterial reverse mutation assays using *S.typhimurium* strains TA97, TA94, TA98, TA100, TA1535, and TA1537 at 5 mg/plate (with metabolic activation) and in TA 97 and TA 102 at 15 mg/plate (with or without metabolic activation;). A chromosomal test with Chinese hamster lung (CHL) cells revealed equivocal effects on polyploidy at 15 mg/mL

(Ishidate et al., 1984; Fujita et al., 1994; OECD, 2003). These results are supported by studies on disodium succinate hexahydrate.

The available experimental data indicate that 2-butoxyethan-1-ol is not genotoxic.

For the remaining candidate substances, the genotoxic potential cannot be assessed adequately, however, from the limited data available there were no indications that genotoxicity for these substances should give rise to safety concern.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

The 61 candidate substances are alcohols, aldehydes, acetals, carboxylic acids and esters containing additional oxygenated functional groups and lactones.

Thirty-five of the 61 candidate substances possess one or more chiral centres and eight can exist as geometrical isomers due to the presence and the position of a double bond. For four of these substances [FL-no: 10.038, 10.040, 10.059 and 10.063] the stereoisomeric composition/composition of mixture has not been specified sufficiently.

Fifty-four of the candidate substances belong to structural class I, six of the candidate substances belong to structural class II, and one belongs to structural class III according to the decision tree approach presented by Cramer et al. (1978).

Forty-eight of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

The candidate substances which have been assigned to structural class I have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 1500 microgram. The candidate substances from structural class II have MSDIs ranging from 0.0012 to 1.2 microgram and the one candidate substance assigned to structural class III has an estimated European daily *per capita* intake of 0.011 microgram (Table 6.1). These intakes are below the thresholds of concern of 1800, 540 and 90 microgram/person/day for structural class I , II and III, respectively.

According to the default MSDI approach, the 61 flavouring substances in this group to which the Procedure has been applied have intakes in Europe from 0.0012 to 48 microgram/*capita*/day which are below the thresholds of concern value for structural class I, II and III substances of 1800, 540 and 90 microgram/person/day, respectively.

The combined estimated daily *per capita* intake as flavourings of the 54 candidate substances assigned to structural class I is 1600 microgram, which does not exceed the threshold of concern for a substance belonging to structural class I of 1800 microgram/person/day. Likewise, the combined estimated daily *per capita* intake as flavouring of the six candidate substances assigned to structural class II is 1.2 microgram, which does not exceed the threshold of concern for a substance belonging to structural class II of 540 microgram/person/day.

The 14 candidate lactones are structurally related to 27 supporting lactones from structural class I, for which the combined intake based on the MSDI approach is approximately 20000 microgram/*capita*/day. The supporting substances were evaluated by the JECFA at the 49th meeting, where it was noted that although the combined intake exceeds the threshold for the structural class, the lactones are expected to be hydrolysed and completely metabolised to innocuous products at the estimated level of intake as flavouring substances, and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the additional intake of about

5 microgram/*capita*/day for the candidate lactones is negligible compared to the combined intake of 20000 microgram/*capita*/day of the supporting lactones.

Likewise 40 candidate substances are structurally related to 32 supporting aliphatic primary alcohols and related substances containing an additional oxygenated functional group from structural class I, and for which intake data are available. The combined intake of these supporting substances amounts to approximately 25000 microgram/*capita*/day based on the MSDI approach. These substances were evaluated at the 53rd JECFA meeting, where it was also noted that the substances are expected to be efficiently metabolised to innocuous products and would not give rise to perturbations outside the physiological range. The Panel agreed with this view and concluded that the contribution from the combined intake of the candidate substances of 1540 microgram/*capita*/day would not alter the JECFA conclusion based on a combined intake of 25000 microgram/*capita*/day.

On the basis of the data available it is concluded that there is no indication that the candidate substances in the present Flavouring Group Evaluation possess genotoxic potential. However, the Panel reconsidered the fact that 1-hydroxypropan-2-one [FL-no: 07.169] is an endogenous metabolite of acetone. Acetone is endogenously formed from the degradation of body fat/fatty acids and occurs in the blood of healthy humans not exposed to external sources of acetone in amounts of approximately 4 - 12 mg/person corresponding to 0.7 to 2 mg/l blood. Under these conditions, the majority of the acetone in blood would be metabolised to 1-hydroxypropan-2-one, which is rapidly further metabolised to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway. The estimated exposure of 0.22 microgram/*capita*/day is considerably lower than that resulting from the metabolism of acetone and would not significantly add to the internal exposure to 1-hydroxypropan-2-one in the body and would not perturb the normal catabolism of the compound to innocuous endogenous products. The Panel therefore decided that further genotoxicity data are not required and that the substance could be taken through the Procedure.

It can be anticipated that, at the estimated levels of intake as flavouring substances, the alcohols, aldehydes, acetals, carboxylic acids and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products, many of which are endogenous in humans. The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351], as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structurally related diethyl fumarate [FL-no: 09.350]. However, as the estimated levels of intake as flavouring substances are sufficiently low for these two substances, profound GSH depletion is not expected. For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are, 2-butoxyethanol [FL-no: 02.242], the major metabolite of which butoxyacetic acid has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol, 1,1,3-triethoxypropane [FL-no: 06.097], which may be metabolised to 3-ethoxypropanoic acid, a substance which has structural similarities to 2-butoxyethanol and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], of which hydrolysis gives rise to 2-acetylbutyric acid, which shows some structural similarities to valproic acid, a known teratogenic compound. Adequate margins of safety could be established for these three substances in step B4 of the Procedure.

Otherwise, it was noted that where toxicity data were available they were consistent with the conclusions in the present Flavouring Group Evaluation using the Procedure.

It was considered that on the basis of the default MSDI approach the 61 flavouring substances, to which the Procedure have been applied, would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

The mTAMDI for the 53 candidate substances in structural class I, for which use levels information is available, range from 1600 to 5100 microgram/person/day. For 51 of these substances the mTAMDI is above the threshold of concern of 1800 microgram/person/day.

The mTAMDI of the five substances assigned to structural class II, and for which use levels information is available, range from 3800 to 3900 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day.

For the one substance from structural class III the mTAMDI is 4100, which is above the threshold of 90 microgram/person/day.

Thus, for 59 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For two substances [FL-no: 06.135 and 08.113] no use levels have been provided for the food categories as listed in Commission Regulation (EC) No 1565/2000. The two candidate substances [FL-no: 05.149 and 07.169] which have mTAMDI intake estimates below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether the conclusion for the 61 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for 55 flavouring substances. For two substances [FL-no: 06.135 and 08.113] information on solubility is lacking. For four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] information on composition of mixture and/or stereoisomerism has not been specified sufficiently. For one substance [FL-no: 10.063] is an identity test missing. Thus, the final evaluation of the materials of commerce cannot be performed for four substances [FL-no: 10.038, 10.040, 10.059 and 10.063] pending further information.

For the remaining 57 candidate substances [FL-no: 02.132, 02.198, 02.242, 05.149, 06.088, 06.090, 06.095 06.097, 06.102, 06.135, 07.169, 08.053, 08.082, 08.090, 08.103, 08.113, 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874, 09.916, 10.039, 10.045, 10.047 - 10.049, 10.052, 10.055, 10.058, 10.068 and 10.168] the Panel concluded that they would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 10, REVISION 2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

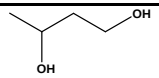
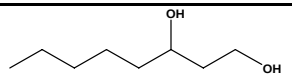
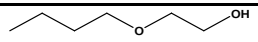
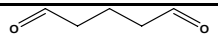
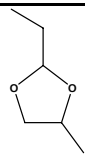
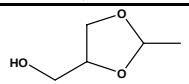
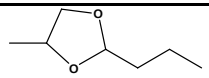
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
02.132	Butane-1,3-diol		107-88-0	Liquid $C_4H_{10}O_2$ 90.12	Soluble Freely soluble	102 (13 hPa) MS 95 %	1.436-1.442 0.992-0.998	Racemate.
02.198	Octane-1,3-diol		23433-05-8	Liquid $C_8H_{18}O_2$ 146.23	Sparingly soluble Freely soluble	82 (7 hPa) MS 95 %	1.452-1.458 0.980-0.986	Racemate.
02.242	2-Butoxyethan-1-ol		10182 111-76-2	Liquid $C_6H_{14}O_2$ 118.18	Slightly soluble Freely soluble	170 MS 95 %	1.416-1.422 0.899-0.905	
05.149	Glutaraldehyde		111-30-8	Liquid $C_5H_8O_2$ 100.12	Soluble Freely soluble	188 MS 95 %	1.430-1.436 1.005-1.011	
06.088	2-Ethyl-4-methyl-1,3-dioxolane		4359-46-0	Liquid $C_6H_{12}O_2$ 116.16	Soluble Freely soluble	116 MS 95 %	1.402-1.408 0.916-0.922	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		3674-21-3	Liquid $C_5H_{10}O_3$ 118.13	Practically insoluble or insoluble Freely soluble	187 MS 95 %	1.440-1.446 1.120-1.126	Racemate. CASrn in Register to be changed to 3773-93-1 (EFFA) CASrn in Register refers to the (2R, 4S) enantiomer.
06.095	4-Methyl-2-propyl-1,3-dioxolane		4352-99-2	Liquid $C_7H_{14}O_2$ 130.19	Soluble Freely soluble	143 MS 95 %	1.409-1.415 0.907-0.913	ID 7). Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a). ID test is missing.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

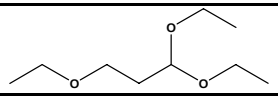
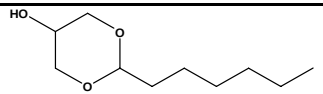
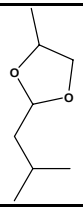
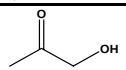
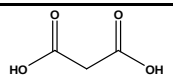
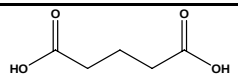
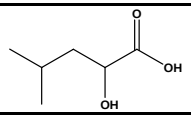
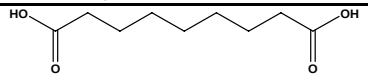
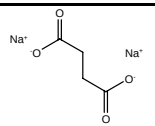
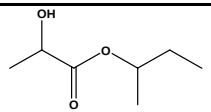
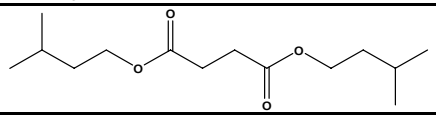
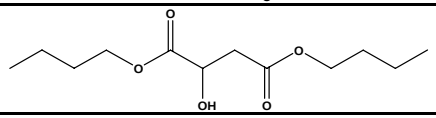
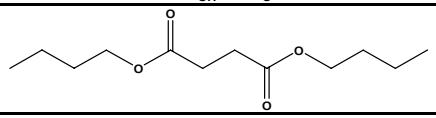
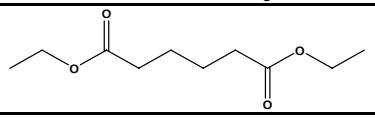
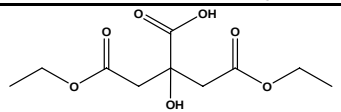
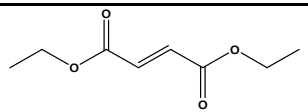
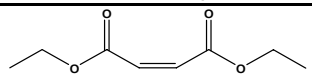
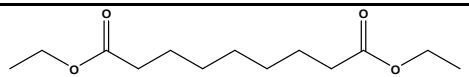
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
06.097	1,1,3-Triethoxypropane		10075 7789-92-6	Liquid $C_9H_{20}O_3$ 176.26	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.403-1.409 0.890-0.896	
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		2016 1708-36-7	Solid $C_{10}H_{20}O_3$ 188.22	Practically insoluble or insoluble Freely soluble	255 44 MS 95 %	n.a. n.a.	
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		4378 18433-93-7	Liquid $C_8H_{16}O_2$ 144.21		150 MS 96 %	n.a. 0.895	SE 8), SW 9). Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).
07.169	1-Hydroxypropan-2-one		11101 116-09-6	Liquid $C_3H_6O_2$ 74.08	Soluble Freely soluble	146 MS 95 %	1.420-1.426 1.084-1.090	
08.053	Malonic acid		2264 141-82-2	Solid $C_3H_4O_4$ 104.16	Soluble Freely soluble	264 135 MS 95 %	n.a. n.a.	
08.082	Glutaric acid		110-94-1	Solid $C_5H_8O_4$ 132.12	Soluble Freely soluble	303 98 MS 95 %	n.a. n.a.	
08.090	2-Hydroxy-4-methylvaleric acid		10118 498-36-2	Solid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	249 76 MS 95 %	n.a. n.a.	Racemate.
08.103	Nonanedioic acid		10079 123-99-9	Solid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	225 (13 hPa) 107 MS 95 %	n.a. n.a.	
08.113	Succinic acid, disodium salt		3277 150-90-3	Solid $C_4H_4Na_2O_4$ 162.05		426.03 156.43 IR 60	n.a. n.a.	SE 8), SW 9). Anhydrous when heated to 120°C. Min.assay: Anhydrous 60 %, hydrate 40 %

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
								(Fenaroli).
09.333	sec-Butyl lactate		18449-60-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	172 MS 95 %	1.414-1.420 0.970-0.976	Racemate.
09.345	Di-isopentyl succinate		10555 818-04-2	Liquid $C_{14}H_{26}O_4$ 258.36	Practically insoluble or insoluble Freely soluble	298 MS 95 %	1.431-1.437 0.955-0.961	
09.346	Dibutyl malate		1587-18-4	Solid $C_{12}H_{22}O_5$ 246.30	Practically insoluble Freely soluble	170 (16 hPa) 82 MS 95 %	n.a. n.a.	CASrn in Register to be changed to 6280-99-5 (racemate).
09.347	Dibutyl succinate		141-03-7	Liquid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	275 MS 95 %	1.426-1.432 0.973-0.979	
09.348	Diethyl adipate		141-28-6	Liquid $C_{10}H_{18}O_4$ 202.25	Practically insoluble or insoluble Freely soluble	244 MS 95 %	1.425-1.431 1.004-1.010	
09.349	Diethyl citrate		32074-56-9	Solid $C_{10}H_{16}O_7$ 248.23	Sparingly soluble Freely soluble	354 237 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register refers to incompletely defined substance.
09.350	Diethyl fumarate		623-91-6	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.444 1.049-1.055	
09.351	Diethyl maleate		10551 141-05-9	Liquid $C_8H_{12}O_4$ 172.18	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.438-1.445 1.049-1.055	
09.352	Diethyl nonanedioate		10549 624-17-9	Liquid $C_{13}H_{24}O_4$ 244.33	Practically insoluble or insoluble Freely soluble	290 NMR 95 %	1.432-1.438 0.970-0.976	

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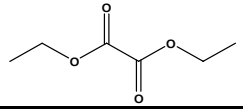
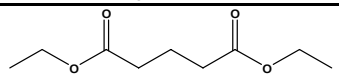
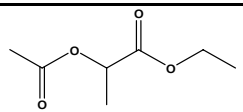
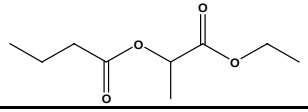
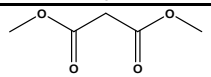
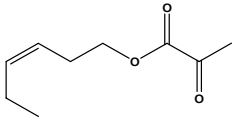
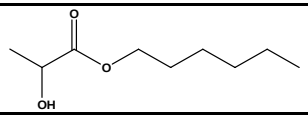
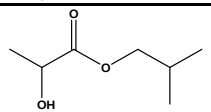
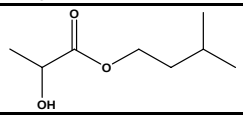
09.353	Diethyl oxalate		95-92-1	Liquid $C_6H_{10}O_4$ 146.14	Practically insoluble or insoluble Freely soluble	185 MS 95 %	1.407-1.413 1.076-1.082	
09.354	Diethyl pentanedioate		818-38-2	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	233 MS 95 %	1.421-1.427 1.019-1.025	
09.360	Ethyl 2-acetoxypropionate		2985-28-6	Liquid $C_7H_{12}O_4$ 160.17	Practically insoluble or insoluble Freely soluble	76 (13 hPa) MS 95 %	1.405-1.411 1.041-1.047	Racemate.
09.502	Ethyl butyryl lactate		2242 71662-27-6	Liquid $C_9H_{16}O_4$ 188.22	Sparingly soluble Freely soluble	208 MS 95 %	1.408-1.414 1.021-1.027	Racemate.
09.558	Dimethyl malonate		11754 108-59-8	Liquid $C_5H_8O_4$ 132.12	Practically insoluble or insoluble Freely soluble	181 MS 95 %	1.411-1.417 1.150-1.156	
09.565 1846	Hex-3-enyl 2-oxopropionate		3934 10684 68133-76-6	Liquid $C_9H_{14}O_3$ 170.21	Practically insoluble or insoluble Freely soluble	76 (0.7 hPa) IR NMR 98 %	1.437-1.445 0.982-0.990	Register name to be changed to Hex-(3Z)-enyl 2-oxopropionate (EFFA, 2010a).
09.580	Hexyl lactate		20279-51-0	Liquid $C_9H_{18}O_3$ 174.24	Slightly soluble Freely soluble	221 MS 95 %	1.426-1.432 0.951-0.957	Racemate.
09.590	Isobutyl lactate		10709 585-24-0	Liquid $C_7H_{14}O_3$ 146.19	Slightly soluble Freely soluble	182 MS 95 %	1.415-1.421 0.968-0.974	Racemate.
09.601	Isopentyl lactate		10720 19329-89-6	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	202 MS 97 %	1.421-1.427 0.958-0.974	Racemate.

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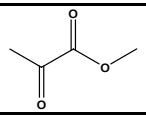
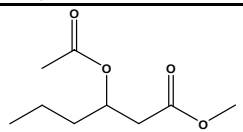
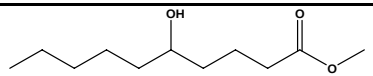
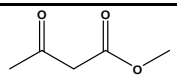
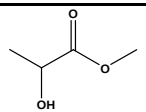
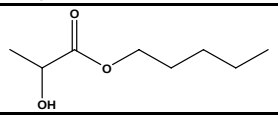
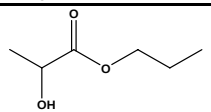
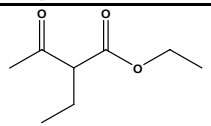
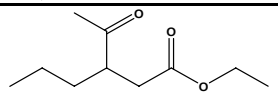
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.626	Methyl 2-oxopropionate		10848 600-22-6	Liquid $C_4H_6O_3$ 120.09	Sparingly soluble Freely soluble	137 MS 95 %	1.401-1.407 1.145-1.151	
09.629	Methyl 3-acetoxyhexanoate		10755 77118-93-5	Liquid $C_9H_{16}O_4$ 188.22	Practically insoluble or insoluble Freely soluble	55 (0.7 hPa) MS 95 %	1.420-1.426 1.013-1.019	Racemate. CASrn in Register to be changed to 21188-60-3 CASrn in Register refers to the (R) enantiomer.
09.633	Methyl 5-hydroxydecanoate		101853-47-8	Solid $C_{11}H_{22}O_3$ 202.29	Practically insoluble or insoluble Freely soluble	278 28 MS 95 %	n.a. n.a.	Racemate.
09.634	Methyl acetoacetate		105-45-3	Liquid $C_5H_8O_3$ 116.12	Sparingly soluble Freely soluble	169 28 MS 95 %	1.415-1.421 1.073-1.079	
09.644	Methyl lactate		27871-49-4	Liquid $C_4H_8O_3$ 104.10	Sparingly soluble Freely soluble	244 MS 95 %	1.408-1.414 1.060-1.066	Register name to be changed to (S)-Methyl lactate.
09.683	Pentyl lactate		6382-06-5	Liquid $C_8H_{16}O_3$ 160.21	Slightly soluble Freely soluble	206 MS 95 %	1.423-1.429 0.965-0.971	Racemate.
09.815	Propyl lactate		616-09-1	Liquid $C_6H_{12}O_3$ 132.16	Sparingly soluble Freely soluble	170 MS 95 %	1.414-1.420 1.000-1.006	Racemate.
09.824	Ethyl 2-acetylbutyrate		607-97-6	Liquid $C_8H_{14}O_3$ 158.20	Practically insoluble or insoluble Freely soluble	198 MS 95 %	1.417-1.423 0.982-0.988	Racemate.
09.832	Ethyl 3-acetohexanoate		10566 21188-61-4	Liquid $C_{10}H_{18}O_3$ 186.24	Practically insoluble or insoluble Freely soluble	110 (12 hPa) MS 95 %	1.419-1.425 1.009-1.015	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

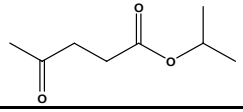
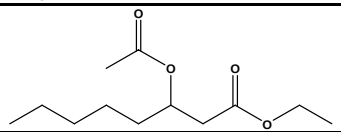
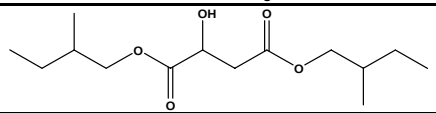
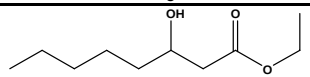
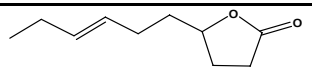
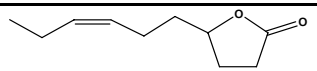
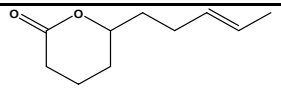
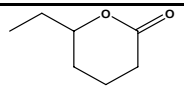
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
09.833	iso-Propyl 4-oxopentanoate		21884-26-4	Liquid $C_8H_{14}O_3$ 158.20	Sparingly soluble Freely soluble	209 MS 95 %	1.418-1.424 0.981-0.987	
09.862	Ethyl 3-acetoxy octanoate		85554-66-1	Solid $C_{12}H_{22}O_4$ 230.30	Practically insoluble or insoluble Freely soluble	276 21 MS 95 %	n.a. n.a.	Racemate.
09.874	Di(2-methylbutyl) malate			Solid $C_{14}H_{26}O_5$ 274.35	Sparingly soluble Freely soluble	335 74 NMR 95 %	n.a. n.a.	Racemate. CASrn in Register to be introduced 253596-99-5.
09.916	Ethyl 3-hydroxyoctanoate		10603 7367-90-0	Liquid $C_{10}H_{20}O_3$ 188.27	Practically insoluble or insoluble Freely soluble	118 (12 hPa) MS 95 %	1.421-1.427 0.973-0.979	Racemate (EFFA, 2010a).
10.038	Dec-7-eno-1,4-lactone		67114-38-9	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a) Composition of mixture to be specified.
10.039	cis-Dec-7-eno-1,4-lactone		63095-33-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	165 (0.3 hPa) MS 95 %	1.462-1.468 0.974-0.980	Racemate
10.040	Dec-8-eno-1,5-lactone		32764-98-0	Liquid $C_{10}H_{16}O_2$ 168.24	Practically insoluble or insoluble Freely soluble	157 (15 hPa) MS 95 %	1.462-1.468 0.972-0.978	Racemate, mixture of (Z)- and (E)-isomers (EFFA, 2010a) Composition of mixture to be specified.
10.045	Heptano-1,5-lactone		10660 3301-90-4	Liquid $C_7H_{12}O_2$ 128.17	Practically insoluble or insoluble Freely soluble	104 (12 hPa) MS 95 %	1.451-1.457 1.031-1.037	Racemate.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

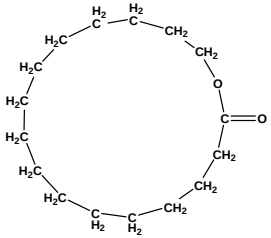
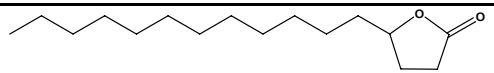
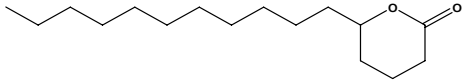
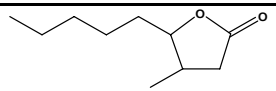
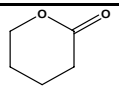
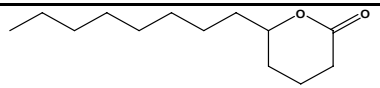
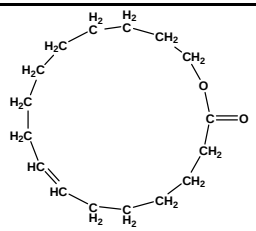
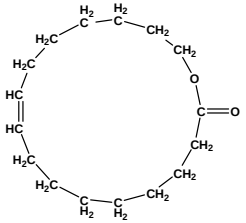
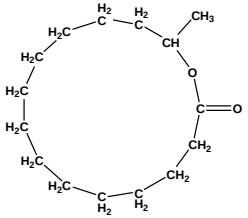
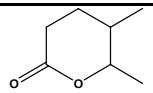
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.047	Hexadecano-1,16-lactone		109-29-5	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	128 (1 hPa) 34 MS 95 %	n.a. n.a.	
10.048	Hexadecano-1,4-lactone		10673 730-46-1	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	185 (5 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.049	Hexadecano-1,5-lactone		10674 7370-44-7	Solid $C_{16}H_{30}O_2$ 254.41	Practically insoluble or insoluble Freely soluble	130 (1 hPa) 38 MS 95 %	n.a. n.a.	Racemate.
10.052	3-Methylnonano-1,4-lactone		33673-62-0	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	115 (3 hPa) MS 95 %	1.444-1.450 0.945-0.951	Racemate.
10.055	Pentano-1,5-lactone		10907 542-28-9	Liquid $C_5H_8O_2$ 100.12	Sparingly soluble Freely soluble	219 MS 95 %	1.451-1.457 1.101-1.107	
10.058	Tridecano-1,5-lactone		10902 7370-92-5	Liquid $C_{13}H_{24}O_2$ 212.33	Practically insoluble or insoluble Freely soluble	188 (15 hPa) MS 95 %	1.455-1.463 0.939-0.953	Racemate.
10.059	Hexadec-7-en-1,16-lactone 6)		123-69-3	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	188 (20 hPa) MS 95 %	1.482-1.488 0.955-0.961	CASrn in Register refers to the Z-isomer. Stereoisomeric composition to be specified.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 10, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
10.063	Hexadec-9-en-1,16 lactone 6)		28645-51-4	Liquid $C_{16}H_{28}O_2$ 252.40	Practically insoluble or insoluble Soluble	131 (0.9 hPa) 95 %	1.476-1.482 0.953-0.959	ID 7). CASrn in Register does not specify isomeric composition.. Stereoisomeric composition to be specified.
10.068	Pentadecano-1,14-lactone		32539-85-8	Liquid $C_{15}H_{28}O_2$ 240.38	Practically insoluble or insoluble Freely soluble	108 (0.1 hPa) MS 95 %	1.466-1.472 0.942-0.948	Racemate.
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		4141 10413-18-0	Liquid $C_7H_{12}O_2$ 128.17	Slightly soluble Freely soluble	60 NMR MS 98 %	1.452-1.458 1.019-1.025	Mixture of ((R/R), (R/S), (S/R) & (S/S) in equal ratios) (EFFA, 2010a).

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.
7) ID: Missing identification test.
8) SE: Missing data on solubility in ethanol.
9) SW: Missing data on solubility.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

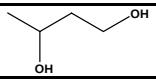
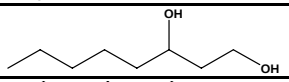
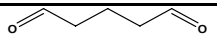
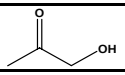
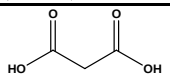
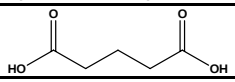
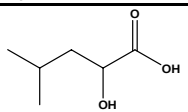
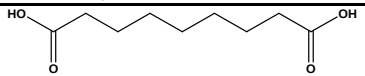
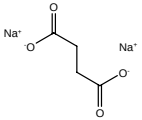
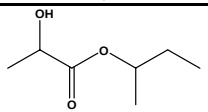
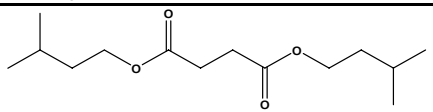
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
02.132	Butane-1,3-diol		0.0061	Class I A3: Intake below threshold	4)	6)	a)
02.198	Octane-1,3-diol		0.0012	Class I A3: Intake below threshold	4)	6)	a)
05.149	Glutaraldehyde		0.055	Class I A3: Intake below threshold	4)	6)	a)
07.169	1-Hydroxypropan-2-one		0.22	Class I A3: Intake below threshold	4)	6)	a)
08.053	Malonic acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.082	Glutaric acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.090	2-Hydroxy-4-methylvaleric acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.103	Nonanedioic acid		0.0012	Class I A3: Intake below threshold	4)	6)	a)
08.113	Succinic acid, disodium salt		1500	Class I A3: Intake below threshold	4)	6)	a)
09.333	sec-Butyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	a)
09.345	Di-isopentyl succinate		0.037	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

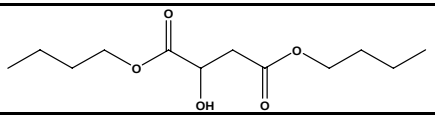
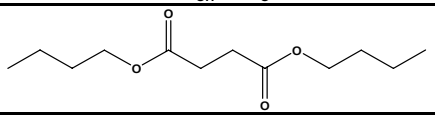
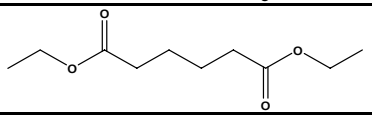
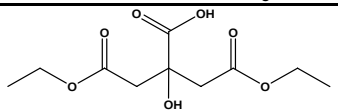
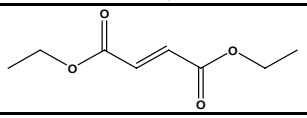
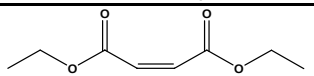
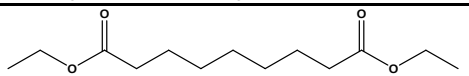
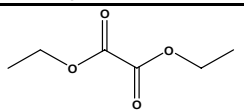
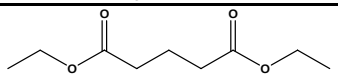
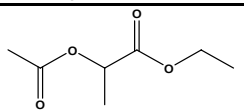
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.346	Dibutyl malate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.347	Dibutyl succinate		0.12	Class I A3: Intake below threshold	4)	6)	a)
09.348	Diethyl adipate		0.027	Class I A3: Intake below threshold	4)	6)	a)
09.349	Diethyl citrate		0.12	Class I A3: Intake below threshold	4)	6)	a)
09.350	Diethyl fumarate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.351	Diethyl maleate		12	Class I A3: Intake below threshold	4)	6)	a)
09.352	Diethyl nonanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.353	Diethyl oxalate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.354	Diethyl pentanedioate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.360	Ethyl 2-acetoxypropionate		4.9	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

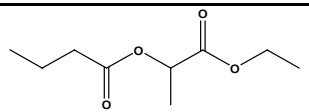
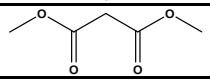
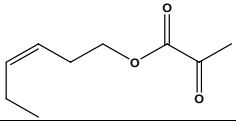
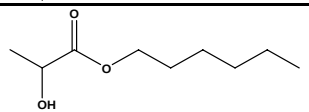
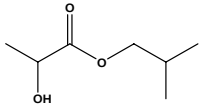
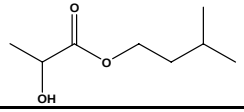
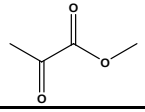
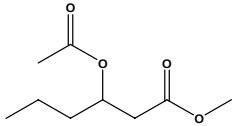
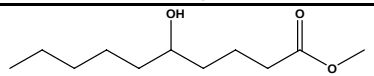
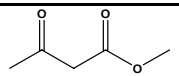
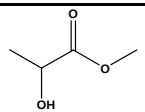
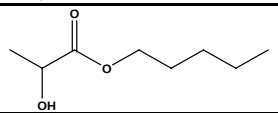
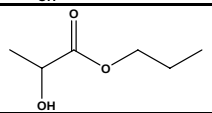
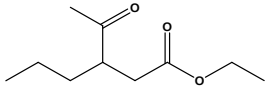
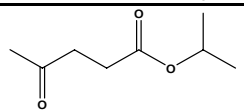
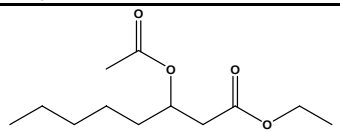
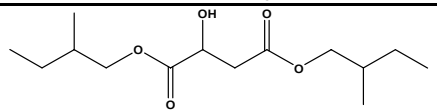
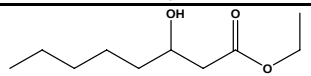
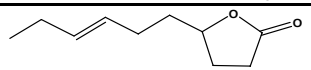
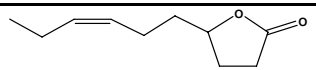
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.502	Ethyl butyryl lactate		0.5	Class I A3: Intake below threshold	4)	6)	a)
09.558	Dimethyl malonate		0.097	Class I A3: Intake below threshold	4)	6)	a)
09.565 1846	Hex-3-enyl 2-oxopropionate		0.74	Class I A3: Intake below threshold	4)	6)	a)
09.580	Hexyl lactate		0.49	Class I A3: Intake below threshold	4)	6)	a)
09.590	Isobutyl lactate		3.7	Class I A3: Intake below threshold	4)	6)	a)
09.601	Isopentyl lactate		7.2	Class I A3: Intake below threshold	4)	6)	a)
09.626	Methyl 2-oxopropionate		0.024	Class I A3: Intake below threshold	4)	6)	a)
09.629	Methyl 3-acetoxyhexanoate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.633	Methyl 5-hydroxydecanoate		0.24	Class I A3: Intake below threshold	4)	6)	a)
09.634	Methyl acetoacetate		0.012	Class I A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
09.644	Methyl lactate		0.34	Class I A3: Intake below threshold	4)	6)	a)
09.683	Pentyl lactate		0.61	Class I A3: Intake below threshold	4)	6)	a)
09.815	Propyl lactate		0.62	Class I A3: Intake below threshold	4)	6)	a)
09.832	Ethyl 3-acetohexanoate		0.33	Class I A3: Intake below threshold	4)	6)	a)
09.833	iso-Propyl 4-oxopentanoate		0.24	Class I A3: Intake below threshold	4)	6)	a)
09.862	Ethyl 3-acetoxy octanoate		0.0012	Class I A3: Intake below threshold	4)	6)	a)
09.874	Di(2-methylbutyl) malate		0.015	Class I A3: Intake below threshold	4)	6)	a)
09.916	Ethyl 3-hydroxyoctanoate		0.011	Class I A3: Intake below threshold	4)	6)	a)
10.038	Dec-7-eno-1,4-lactone		0.37	Class I A3: Intake below threshold	4)	7)	a)
10.039	cis-Dec-7-eno-1,4-lactone		1.2	Class I A3: Intake below threshold	4)	6)	a)

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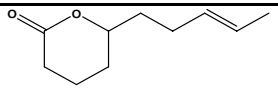
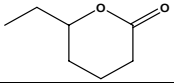
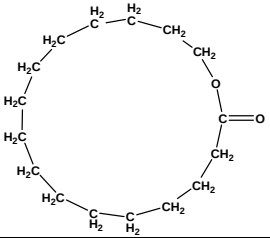
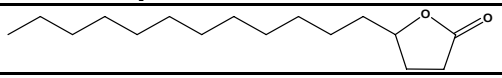
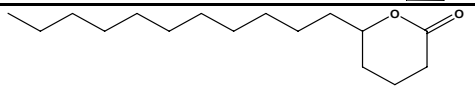
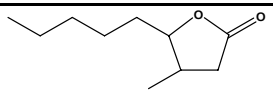
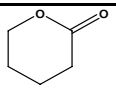
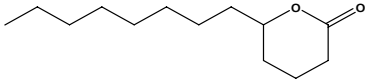
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.040	Dec-8-eno-1,5-lactone		0.011	Class I A3: Intake below threshold	4)	7)	a)
10.045	Heptano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	a)
10.047	Hexadecano-1,16-lactone		0.024	Class I A3: Intake below threshold	4)	6)	a)
10.048	Hexadecano-1,4-lactone		0.0061	Class I A3: Intake below threshold	4)	6)	a)
10.049	Hexadecano-1,5-lactone		0.024	Class I A3: Intake below threshold	4)	6)	a)
10.052	3-Methylnonano-1,4-lactone		0.61	Class I A3: Intake below threshold	4)	6)	a)
10.055	Pentano-1,5-lactone		0.012	Class I A3: Intake below threshold	4)	6)	a)
10.058	Tridecano-1,5-lactone		0.61	Class I A3: Intake below threshold	4)	6)	a)

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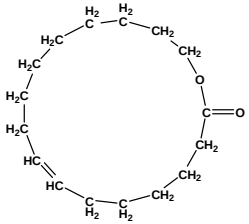
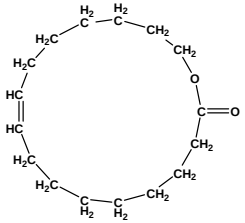
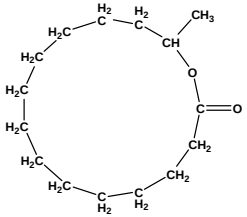
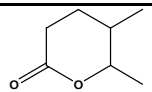
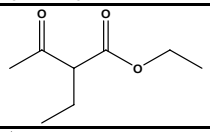
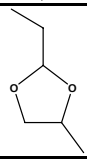
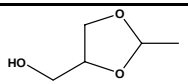
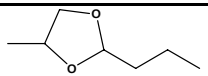
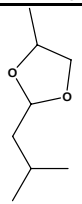
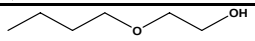
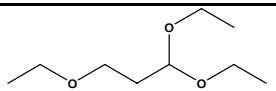
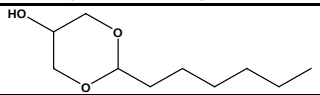
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
10.059	Hexadec-7-en-1,16-lactone		1.9	Class I A3: Intake below threshold	4)	7)	a)
10.063	Hexadec-9-en-1,16 lactone		48	Class I A3: Intake below threshold	4)	7)	a)
10.068	Pentadecano-1,14-lactone		0.9	Class I A3: Intake below threshold	4)	6)	a)
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one		1.2	Class I A3: Intake below threshold	4)	6)	a)
09.824	Ethyl 2-acetylbutyrate		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.088	2-Ethyl-4-methyl-1,3-dioxolane		0.0061	Class II A3: Intake below threshold	4)	6)	a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	a)
06.095	4-Methyl-2-propyl-1,3-dioxolane		0.012	Class II A3: Intake below threshold	4)	6)	a)
06.135 1732	2-Isobutyl-4-methyl-1,3-dioxolane		1.2	Class II A3: Intake below threshold	4)	6)	a)
02.242	2-Butoxyethan-1-ol		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.097	1,1,3-Triethoxypropane		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	a)
06.102	2-Hexyl-5-hydroxy-1,3-dioxane		0.011	Class III A3: Intake below threshold	4)	6)	a)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) No safety concern at the estimated level of intake based on the MSDI approach.

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

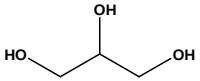
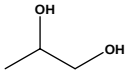
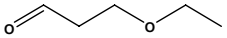
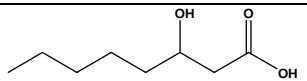
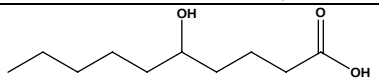
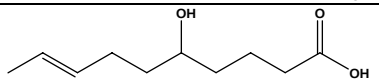
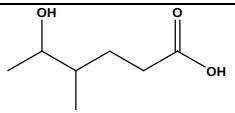
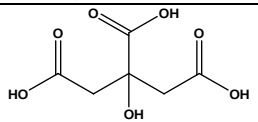
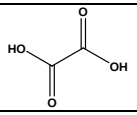
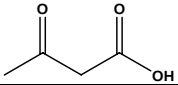
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Glycerol 909		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	Propylene glycol 925		No evaluation Pending definition of "flavouring agent"		Not in EU-Register
	3-Ethoxypropan-1-al		Not evaluated as flavouring substance		Not in EU-Register
	3-Hydroxyoctanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxydecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-8-decenoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	Citric acid		Not evaluated as flavouring substance		Not in EU-Register
	Oxalic acid		Not evaluated as flavouring substance		Not in EU-Register
	Acetoacetic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

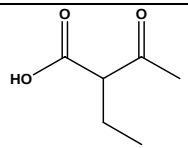
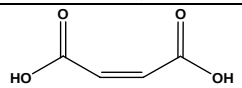
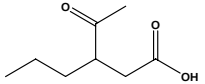
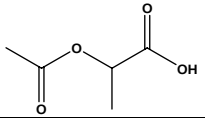
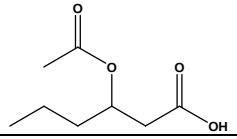
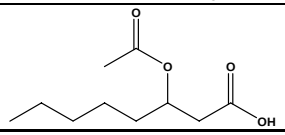
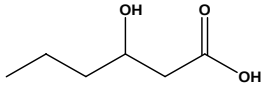
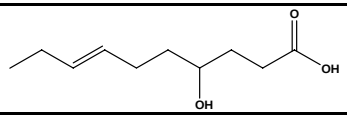
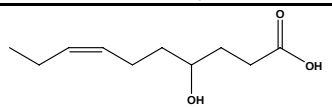
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Acetylbutyric acid		Not evaluated as flavouring substance		Not in EU-Register
	Maleic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetohexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	2-Acetoxypropionic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetoxyhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Acetoxyoctanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	3-Hydroxyhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	(Z)-4-Hydroxydec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

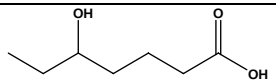
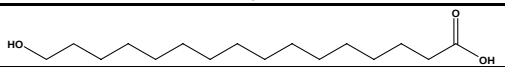
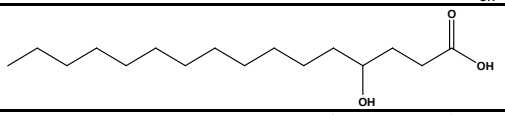
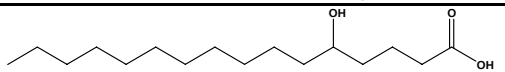
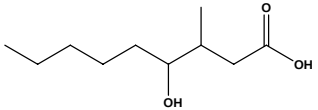
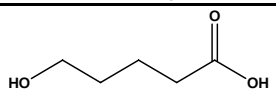
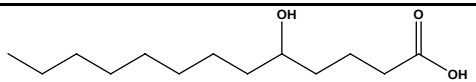
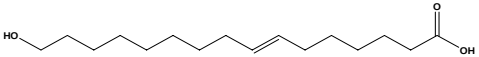
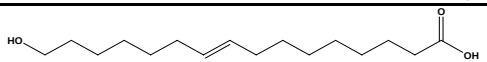
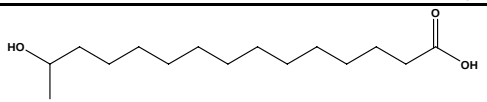
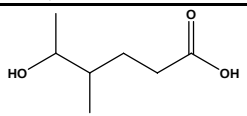
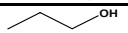
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	5-Hydroxyheptanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxyhexadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	4-Hydroxy-3-methylnonanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxypentanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxytridecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-7-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	16-Hydroxyhexadec-9-enoic acid		Not evaluated as flavouring substance		Not in EU-Register
	14-Hydroxypentadecanoic acid		Not evaluated as flavouring substance		Not in EU-Register
	5-Hydroxy-4-methylhexanoic acid		Not evaluated as flavouring substance		Not in EU-Register
02.002	Propan-1-ol 82		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

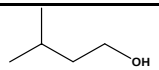
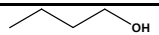
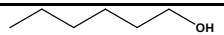
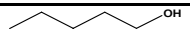
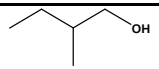
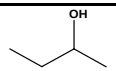
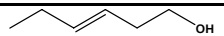
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
02.003	Isopentanol 52		Category 1 a) No safety concern d) Category A c)	Class I A3: Intake below threshold	
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.005	Hexan-1-ol 91		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.040	Pentan-1-ol 88		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
02.076	2-Methylbutan-1-ol 1199		Category 1 a) No safety concern e) Category B c)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a) No safety concern d)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
02.079	Isopropanol 277		Category 1 a) No safety concern f)	Class I A3: Intake above threshold, A4: Endogenous	
02.121	Butan-2-ol		Category 1 a)	No evaluation	
02.159	Hex-3-en-1-ol 315		Category A c)	No evaluation	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

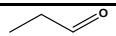
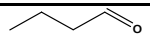
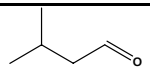
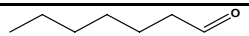
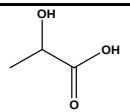
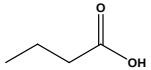
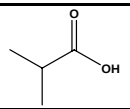
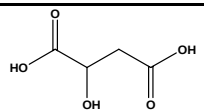
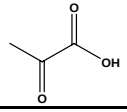
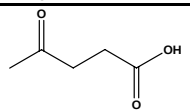
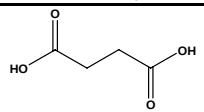
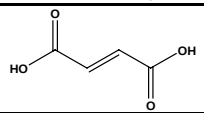
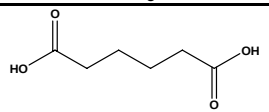
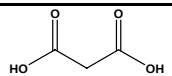
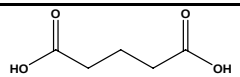
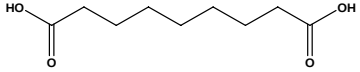
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
05.001	Acetaldehyde 80		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
05.002	Propanal 83		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.003	Butanal 86		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.006	3-Methylbutanal 258		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.031	Heptanal 95		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.004	Lactic acid 930		No safety concern g) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.017	l-Malic acid 619		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.019	Pyruvic acid 936		No safety concern g) Category A c)	Class I A3: Intake below threshold	
08.023	4-Oxovaleric acid 606		No safety concern h) Category A c)	Class I A3: Intake below threshold	
08.024	Succinic acid		Category A c)	No evaluation	
08.025	Fumaric acid 618		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.026	Adipic acid 623		No safety concern h) Category A c)	Class I A3: Intake above threshold, A4: Not endogenous, A5: Adequate NOAEL exists	
08.053	Malonic acid		Category A c) FGE.10	Class I A3: Intake below threshold	
08.082	Glutaric acid		FGE.10	Class I A3: Intake below threshold	
08.103	Nonanedioic acid		FGE.10	Class I A3: Intake below threshold	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.
2) No safety concern at estimated levels of intake.
3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.
4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
a) (SCF, 1995).
b) (JECFA, 1999b).
c) (CoE, 1992).
d) (JECFA, 1997a).
e) (JECFA, 2004a).
f) (JECFA, 2000a).
g) (JECFA, 2002b).
h) (JECFA, 2000b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

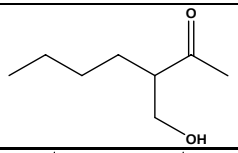
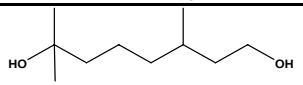
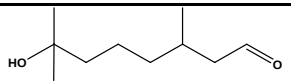
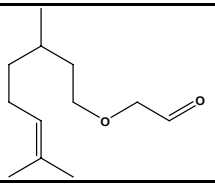
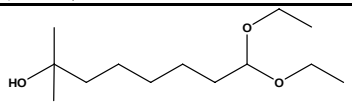
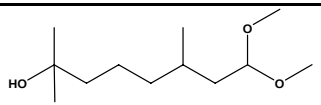
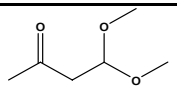
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	3-(Hydroxymethyl)-2-heptanone		2804 592	604 Tentative JECFA spec. (JECFA, 2001c)	7	No safety concern d) Category B	Not in EU-Register.
02.047	3,7-Dimethyloctane-1,7-diol		2586 559 107-74-4	610 JECFA specification (JECFA, 2000d)	9.7	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellol (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
05.012	3,7-Dimethyl-7-hydroxyoctanal		2583 100 107-75-5	611 JECFA specification (JECFA, 1999c)	24	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal (CASrn as in Register). CASrn in Register refers to the racemate.
05.079	Citronellyl oxyacetaldehyde		2310 2012 7492-67-3	592 JECFA specification (JECFA, 2003b)	24	No safety concern a) Category B b)	JECFA evaluated citronelloxyacetaldehyde (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.010	1,1-Diethoxy-3,7-dimethyloctan-7-ol		2584 44 7779-94-4	613 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category B b)	JECFA evaluated hydroxycitronellal diethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.011	1,1-Dimethoxy-3,7-dimethyloctan-7-ol		2585 45 141-92-4	612 JECFA specification (JECFA, 1999c)	0.037	No safety concern a) Category A b)	JECFA evaluated hydroxycitronellal dimethyl acetal (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
06.038	4,4-Dimethoxybutan-2-one		3381 10029 5436-21-5	593 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	

Table 3: Supporting Substances Summary

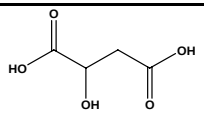
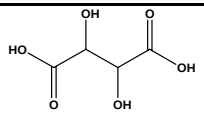
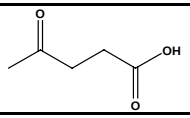
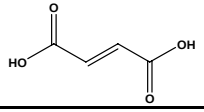
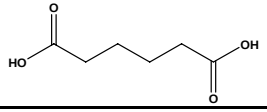
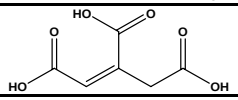
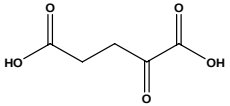
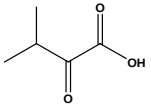
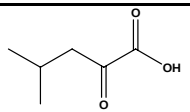
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.017	l-Malic acid		2655 17 6915-15-7	619 JECFA specification (JECFA, 2000d)	13000	No safety concern a) Category A b)	JECFA evaluated l-malic acid (CASrn 97-67-6). (R)- or (S)-enantiomer not specified by CASrn in Register. GrADI: not specified (JECFA, 1970a).
08.018	Tartaric acid		3044 18 133-37-9	621 JECFA specification (JECFA, 1999c)	3800	No safety concern a) Category A b)	JECFA evaluated tartaric acid ((+)-, (-)-, (+/-)-, meso-) (CASrn 87-69-4). CASrn in Register refers to (2R,3R)-isomer. No ADI (JECFA, 1978a).
08.023	4-Oxov aleric acid		2627 23 123-76-2	606 JECFA specification (JECFA, 2002d)	190	No safety concern a) Category A b)	
08.025	Fumaric acid		2488 25 110-17-8	618 JECFA specification (JECFA, 2000d)	780	No safety concern a) Category A b)	GrADI not specified (JECFA, 1990a).
08.026	Adipic acid		2011 26 124-04-9	623 JECFA specification (JECFA, 1999c)	11	No safety concern a) Category A b)	ADI: 0-5 (JECFA, 1978a).
08.033	Prop-1-ene-1,2,3-tricarboxylic acid		2010 33 499-12-7	627 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category A b)	JECFA evaluated aconitic acid (CASrn as in Register). (Z)- or (E)- isomer not specified by CASrn in Register.
08.037	2-Oxoglutaric acid		3891 653 328-50-7	634 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category A b)	
08.051	3-Methyl-2-oxobutyric acid		3869 2262 759-05-7	631 JECFA specification (JECFA, 1999c)	0.012	No safety concern a) Category B b)	JECFA evaluated 3-methyl-2-oxobutanoic acid (the acid and sodium salt) (CASrn as in Register). CASrn in Register refers to the acid.
08.052	4-Methyl-2-oxovaleric acid		3871 2263 816-66-0	633 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category B b)	JECFA evaluated 4-Methyl-2-oxopentanoic acid and its sodium salt (CASrn 816-66-0 and 4502-00-5).

Table 3: Supporting Substances Summary

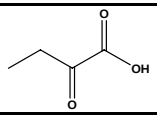
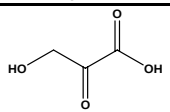
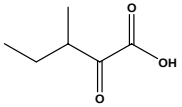
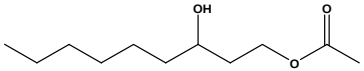
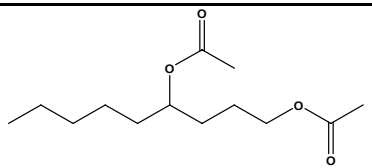
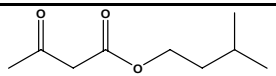
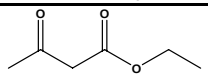
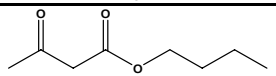
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
08.066	2-Oxobutyric acid		3723 600-18-0	589 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	
08.086	3-Hydroxy-2-oxopropionic acid		3843 1113-60-6	635 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
08.093	3-Methyl-2-oxovaleric acid		3870 10146 39748-49-7	632 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	JECFA evaluated 3-methyl-2-oxopentanoic acid (the acid and sodium salt) (CASrn 1460-34-0). CASrn 39748-49-7 replaced by CASrn 1460-34-0 in the CASrn system (SciFinder). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.225	1,3-Nonanediol acetate		2783 2075 1322-17-4	605 JECFA specification (JECFA, 2005b)	1.8	No safety concern a) Deleted b)	Reg. CASrn refers to incompletely defined substance (mixed esters). Deleted: Substances for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available (CoE, 1992).
09.280	Nonane-1,4-diyl diacetate		3579 11927 67715-81-5	609 JECFA specification (JECFA, 2002d)	0.037	No safety concern a)	JECFA evaluated 1,4-nonanediol diacetate (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
09.401	Isopentyl acetoacetate		3551 227 2308-18-1	598 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.402	Ethyl acetoacetate		2415 240 141-97-9	595 JECFA specification (JECFA, 1999c)	1200	No safety concern a) Category B b)	
09.403	Butyl acetoacetate		2176 241 591-60-6	596 JECFA specification (JECFA, 2000d)	63	No safety concern a) Category B b)	

Table 3: Supporting Substances Summary

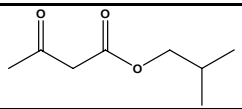
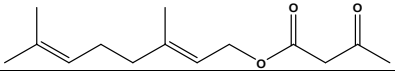
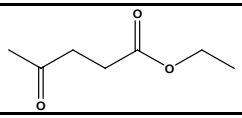
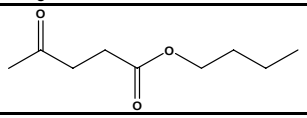
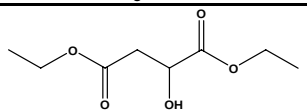
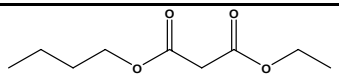
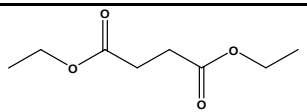
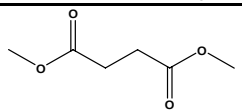
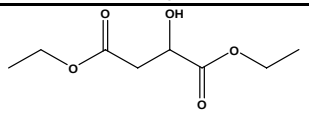
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.404	Isobutyl acetoacetate		2177 242 7779-75-1	597 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
09.405	Geranyl acetoacetate		2510 243 10032-00-5	599 JECFA specification (JECFA, 2001c)	ND	No safety concern a) Category B b)	
09.435	Ethyl 4-oxovalerate		2442 373 539-88-8	607 JECFA specification (JECFA, 1999c)	470	No safety concern a) Category B b)	
09.436	Butyl 4-oxovalerate		2207 374 2052-15-5	608 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
09.439	Diethyl malate		2374 382 7554-12-3	620 JECFA specification (JECFA, 2000d)	3.7	No safety concern a) Deleted b)	JECFA evaluated diethyl malate.CASrn in Register refers to the racemate. Deleted:Substances for which CoE had no information as to their real use in foodstuffs and/or for which insufficient technical and/or toxicological information was available(CoE, 1992).
09.441	Butyl ethyl malonate		2195 384 17373-84-1	615 Tentative JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	
09.444	Diethyl succinate		2377 438 123-25-1	617 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category B b)	
09.445	Dimethyl succinate		2396 439 106-65-0	616 JECFA specification (JECFA, 2002d)	73	No safety concern a) Category B b)	
09.446	Diethyl tartrate		2378 440 87-91-2	622 JECFA specification (JECFA, 2002d)	15	No safety concern a) Category A b)	JECFA evaluated diethyl tartrate (CASrn as in Register). Register CASrn refers to the (2R,3R)-enantiomer. ADI acceptable (JECFA, 2000b).

Table 3: Supporting Substances Summary

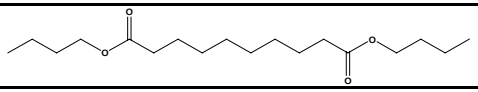
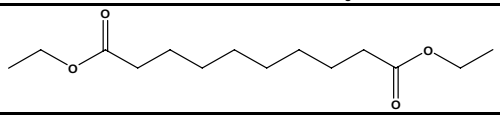
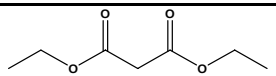
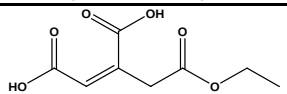
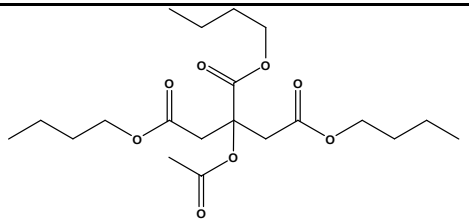
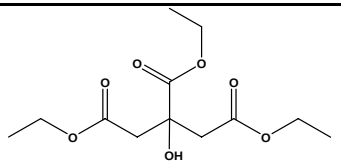
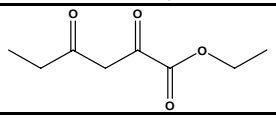
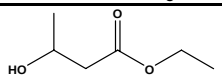
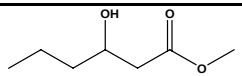
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
09.474	Dibutyl sebacate		2373 622 109-43-3	625 JECFA specification (JECFA, 2003b)	ND	No safety concern a) Category A b)	
09.475	Diethyl sebacate		2376 623 110-40-7	624 JECFA specification (JECFA, 2002d)	120	No safety concern a) Category A b)	
09.490	Diethyl malonate		2375 2106 105-53-3	614 JECFA specification (JECFA, 2002d)	650	No safety concern a) Category A b)	
09.510	Ethyl aconitate		2417 11845 1321-30-8	628 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	JECFA evaluated ethyl aconitate (mixed esters) (CASrn as in Register). Register CASrn refers to incompletely defined substance.
09.511	Tributyl acetylcitrate		3080 77-90-7	630 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
09.512	Triethyl citrate		3083 11762 77-93-0	629 JECFA specification (JECFA, 2000d)	2900	No safety concern a)	ADI: 0-20 (JECFA, 1984a).
09.514	Ethyl 2,4-dioxohexanoate		3278 11903 13246-52-1	603 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
09.522	Ethyl 3-hydroxybutyrate		3428 10596 5405-41-4	594 JECFA specification (JECFA, 2000d)	7.9	No safety concern a)	JECFA evaluated ethyl 3-hydroxybutyrate (CASrn as in Register). Register CASrn refers to the racemate.
09.532	Methyl 3-hydroxyhexanoate		3508 10812 21188-58-9	600 JECFA specification (JECFA, 2000d)	0.85	No safety concern a)	JECFA evaluated methyl 3-hydroxyhexanoate (CASrn as in Register). (R)- or (S)- enantiomer

Table 3: Supporting Substances Summary

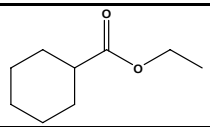
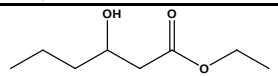
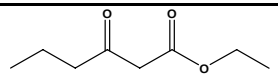
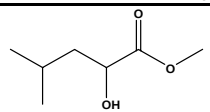
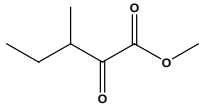
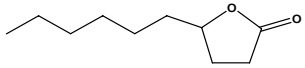
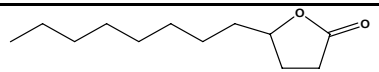
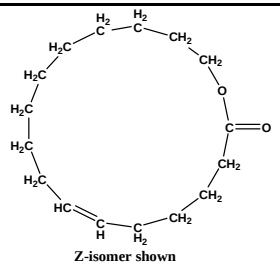
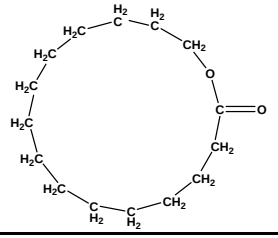
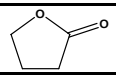
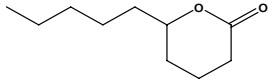
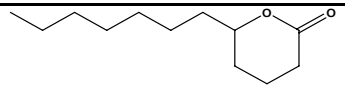
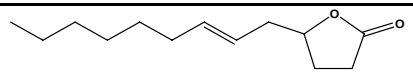
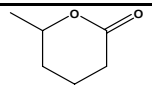
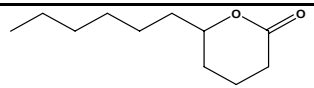
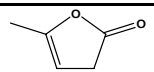
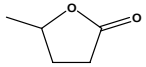
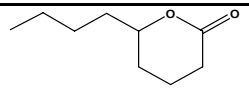
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							not specified by Register CASrn.
09.533	Ethyl brassylate		3543 10571 105-95-3	626 JECFA specification (JECFA, 2002d)	3.0	No safety concern a)	
09.535	Ethyl 3-hydroxyhexanoate		3545 11764 2305-25-1	601 JECFA specification (JECFA, 2002d)	60	No safety concern a)	JECFA evaluated ethyl 3-hydroxyhexanoate (CASrn as in Register). Register CASrn refers to the racemate.
09.542	Ethyl 3-oxohexanoate		3683 3249-68-1	602 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	
09.548	Methyl 2-hydroxy-4-methylvalerate		3706 40348-72-9	590 JECFA specification (JECFA, 2003b)	0.49	No safety concern a)	JECFA evaluated methyl 2-hydroxy-4-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
09.550	Methyl 2-oxo-3-methylvalerate		3713 3682-42-6	591 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	JECFA evaluated methyl 2-oxo-3-methylpentanoate (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.001	Nonano-1,4-lactone		2781 178 104-61-0	229 JECFA specification (JECFA, 2000d)	1000	No safety concern c) Category A b)	JECFA evaluated gamma-nonalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn ADI: 0-1.25 (JECFA, 1968).
10.002	Undecano-1,4-lactone		3091 179 104-67-6	233 JECFA specification (JECFA, 1998b)	1200	No safety concern c) Category A b)	JECFA evaluated gamma-undecalactone (CASrn as in Register). Register CASrn refers to the racemate. ADI: 0-1.25 (JECFA, 1968).
10.003	Hexadec-6-eno-1,16-lactone	 **Z-isomer shown**	2555 180 7779-50-2	240 JECFA specification (JECFA, 2001c)	5.1	No safety concern c) Category B b)	JECFA evaluated omega-6-hexadecenlactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.004	Pentadecano-1,15-lactone		2840 181 106-02-5	239 JECFA specification (JECFA, 2000d)	73	No safety concern c) Category B b)	
10.006	Butyro-1,4-lactone		3291 615 96-48-0	219 JECFA specification (JECFA, 1998b)	110	No safety concern c) Category A b)	
10.007	Decano-1,5-lactone		2361 621 705-86-2	232 JECFA specification (JECFA, 2000d)	7200	No safety concern c) Category B b)	JECFA evaluated delta-decalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.008	Dodecano-1,5-lactone		2401 624 713-95-1	236 JECFA specification (JECFA, 2000d)	5800	No safety concern c) Category B b)	JECFA evaluated delta-dodecalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.009	Dodec-6-eno-1,4-lactone		3780 625 18679-18-0	249 JECFA specification (JECFA, 2001c)	0.012	No safety concern c) Category A b)	JECFA evaluated 1,4-dodec-6-enolactone (CASrn as in Register). Register CASrn refers to the (Z)-isomer.
10.010	Hexano-1,5-lactone		3167 641 823-22-3	224 JECFA specification (JECFA, 1998b)	320	No safety concern c) Category B b)	JECFA evaluated delta-hexalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.011	Undecano-1,5-lactone		3294 688 710-04-3	234 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	JECFA evaluated 5-hydroxyundecanoic acid delta-lactone (CASrn as in Register). Register CASrn refers to the racemate.
10.012	5-Methylfuran-2(3H)-one		3293 731 591-12-8	221 JECFA specification (JECFA, 1998b)	300	No safety concern c) Category B b)	
10.013	Pentano-1,4-lactone		3103 757 108-29-2	220 JECFA specification (JECFA, 1998b)	120	No safety concern c) Category A b)	JECFA evaluted gamma-valerolactone (CASrn as in Register). Register CASrn refers to the racemate.
10.014	Nonano-1,5-lactone		3356 2194 3301-94-8	230 JECFA specification (JECFA, 1998b)	130	No safety concern c) Category B b)	JECFA evaluated hydroxynonanoic acid delta-lactone (CASrn as in Register). Register CASrn refers to the

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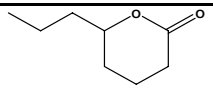
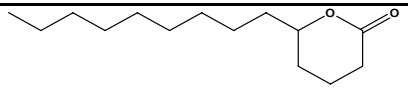
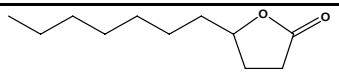
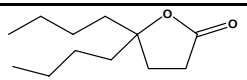
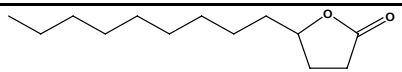
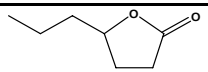
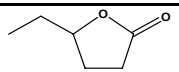
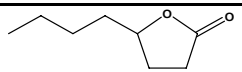
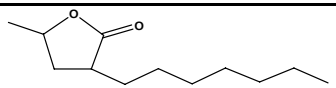
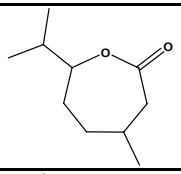
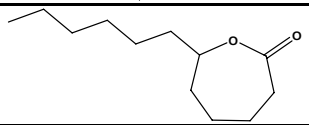
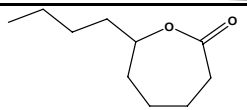
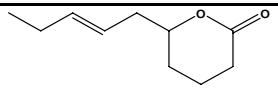
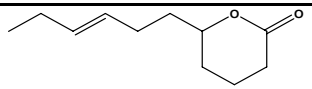
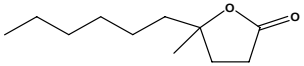
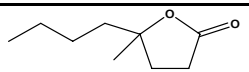
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
							racemate.
10.015	Octano-1,5-lactone		3214 2195 698-76-0	228 JECFA specification (JECFA, 2000d)	230	No safety concern c) Category B b)	JECFA evaluated delta-octalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.016	Tetradecano-1,5-lactone		3590 2196 2721-22-4	238 JECFA specification (JECFA, 1998b)	110	No safety concern c) Category B b)	JECFA evaluated delta-tetradecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.017	Decano-1,4-lactone		2360 2230 706-14-9	231 JECFA specification (JECFA, 1998b)	1600	No safety concern c) Category A b)	JECFA evaluated gamma-decalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.018	4-Butyloctano-1,4-lactone		2372 2231 7774-47-2	227 JECFA specification (JECFA, 2000d)	0.12	No safety concern c) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technological and/or toxicological information was available (CoE, 1992).
10.019	Dodecano-1,4-lactone		2400 2240 2305-05-7	235 JECFA specification (JECFA, 1998b)	190	No safety concern c) Category A b)	JECFA evaluted gamma-dodecalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.020	Heptano-1,4-lactone		2539 2253 105-21-5	225 JECFA specification (JECFA, 2000d)	170	No safety concern c) Category A b)	JECFA evaluated gamma-heptalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.021	Hexano-1,4-lactone		2556 2254 695-06-7	223 JECFA specification (JECFA, 1998b)	160	No safety concern c) Category A b)	JECFA evaluted gamma-hexalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.022	Octano-1,4-lactone		2796 2274 104-50-7	226 JECFA specification (JECFA, 2000d)	430	No safety concern c) Category A b)	JECFA evaluated gamma-octalactone (CASrn as in Register). Register CASrn refers to the racemate.
10.026	3-Heptyldihydro-5-methyl-2(3H)-furanone		3350 10953 40923-64-6	244 JECFA specification (JECFA, 2003b)	0.037	No safety concern c)	JECFA evaluated 3-heptyldihydro-5-methyl-2(3H)-furanone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
10.027	3,7-Dimethyloctano-1,6-lactone		3355 11833 499-54-7	237 JECFA specification (JECFA, 2003b)	0.012	No safety concern c)	JECFA evaluated 6-hydroxy-3,7-dimethyloctanoic acid lactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.028	Dodecano-1,6-lactone		3610 16429-21-3	242 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated epsilon-dodecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.029	Decano-1,6-lactone		3613 5579-78-2	241 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated epsilon-decalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.033	Dec-7-eno-1,5-lactone		3745 34686-71-0	247 JECFA specification (JECFA, 2000d)	0.22	No safety concern c)	JECFA evaluated 5-Hydroxy-7-decenoic acid delta-lactone (CASrn 25524-95-2 which refers to the (Z)-isomer). Neither (Z)- or (E)-isomer nor (R)- or (S)-enantiomer specified by Register CASrn.
10.035	Undec-8-eno-1,5-lactone		3758 68959-28-4	248 JECFA specification (JECFA, 2000d)	0.012	No safety concern c)	JECFA evaluated 5-hydroxy-8-undecenoic acid delta-lactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.
10.051	5-Hexyl-5-methyldihydrofuran-2(3H)-one		3786 7011-83-8	250 JECFA specification (JECFA, 1998b)	ND	No safety concern c)	JECFA evaluated gamma-methyldecalactone (CASrn as in Register). (R)- or (S)- enantiomer not specified by Register CASrn.
10.053	3-Methyloctano-1,4-lactone		3803 10535 39212-23-2	437 JECFA specification (JECFA, 1998b)	ND	No safety concern c)	JECFA evaluated 4-hydroxy-3-methyloctanoic acid gamma-lactone (CASrn as in Register). (R)- or (S)-enantiomer not specified by Register CASrn.

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (JECFA, 2000b).

b) (CoE, 1992).

c) (JECFA, 1999b).

ND) Not determined.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[11] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[12] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[11] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[12] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

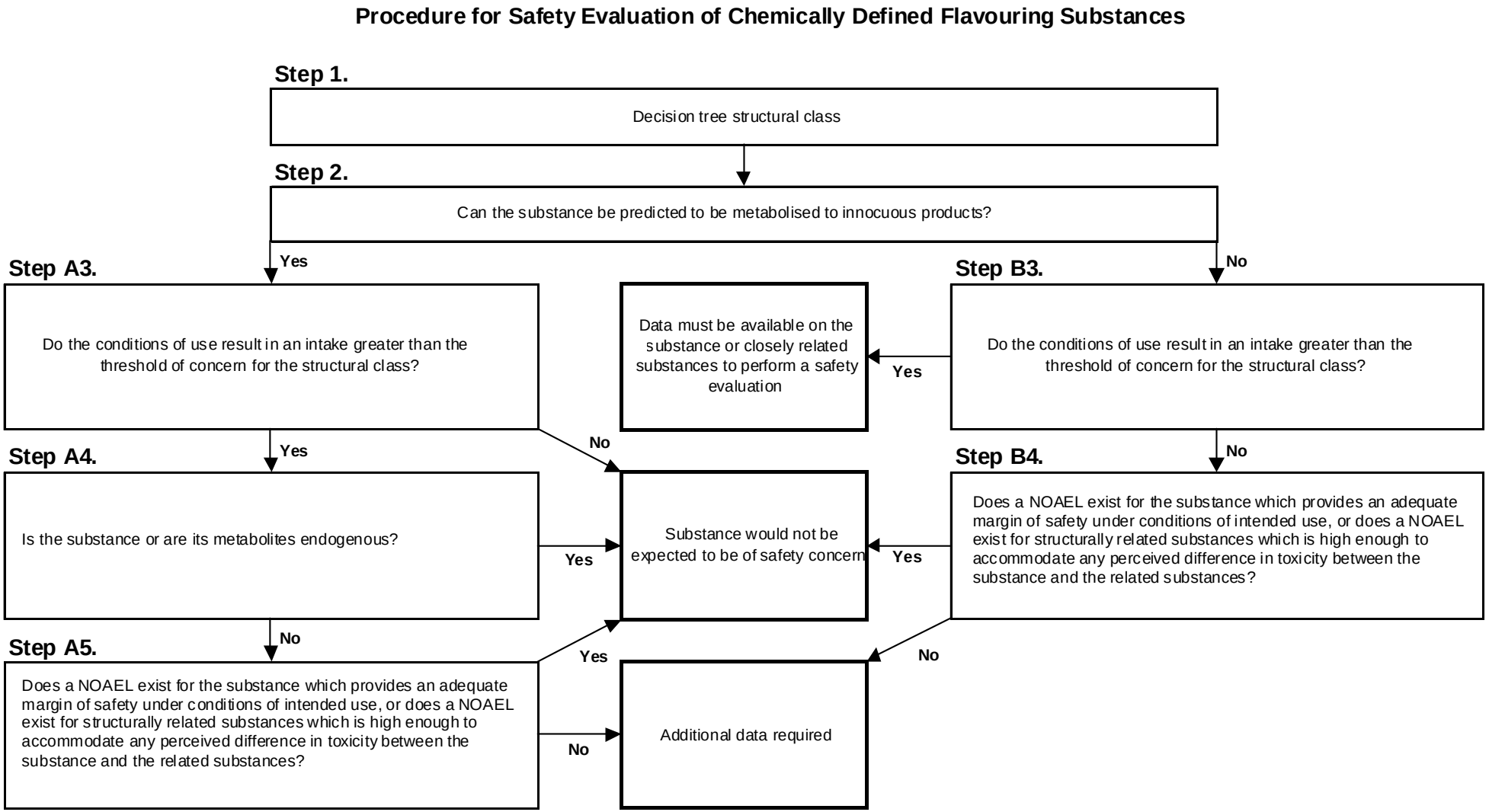


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for 59 of the 61 candidate substances in the present Flavouring Group Evaluatioin (Table II.1.2) (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a).

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
02.132	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.198	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
02.242	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
05.149	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
06.088	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.090	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.095	7	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	100	25
06.097	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
06.102	3	2	3	2	-	10	5	10	2	2	-	-	52	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	5	50	15	50	75	25
07.169	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
08.053	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.082	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
08.090	3	2	3	2	-	10	5	10	2	-	-	-	5	10	5	10	15	5
	15	10	15	10	-	50	25	50	10	-	-	-	25	50	25	50	75	25
08.103	3	2	3	2	-	10	5	10	2	2	-	-	5	10	3	10	15	5
	15	10	15	10	-	50	25	50	10	10	-	-	25	50	15	50	75	25
09.333	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.345	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.346	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.347	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.348	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.349	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.350	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.351	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.352	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.353	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.354	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.360	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.502	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.558	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.565	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.580	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	200	10	10	-	-	25	50	25	50	100	25
09.590	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.601	10	5	10	7	-	20	15	15	2	2	-	-	5	10	5	20	20	5
	50	75	50	35	-	100	75	75	10	10	-	-	25	50	50	100	100	25
09.626	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.629	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.633	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.634	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.644	7	5	10	7	-	10	5	10	2	2	-	-	-	-	5	10	10	5
	35	25	50	35	-	50	25	50	10	10	-	-	-	-	25	50	50	25
09.683	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.815	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.824	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

Table II.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.10

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
09.832	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.833	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.862	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.874	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
09.916	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.038	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.039	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.040	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.045	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.047	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.048	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.049	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.052	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.055	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.058	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.059	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	30	25	50	100	25
10.063	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.068	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
10.168	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	**Distribution of the seven SCF food categories**		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for each of the 59 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2001a; EFFA, 2003c; EFFA, 2003s; EFFA, 2004ag; EFFA, 2007a; Flavour Industry, 2006a). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.132	Butane-1,3-diol	3900	Class I	1800
02.198	Octane-1,3-diol	3900	Class I	1800
05.149	Glutaraldehyde	1600	Class I	1800
07.169	1-Hydroxypropan-2-one	1600	Class I	1800
08.053	Malonic acid	3200	Class I	1800
08.082	Glutaric acid	3200	Class I	1800
08.090	2-Hydroxy-4-methylvaleric acid	3800	Class I	1800
08.103	Nonanedioic acid	3200	Class I	1800
08.113	Succinic acid, disodium salt		Class I	1800
09.333	sec-Butyl lactate	3900	Class I	1800
09.345	Di-isopentyl succinate	3900	Class I	1800
09.346	Dibutyl malate	3900	Class I	1800
09.347	Dibutyl succinate	3900	Class I	1800
09.348	Diethyl adipate	3900	Class I	1800
09.349	Diethyl citrate	3900	Class I	1800
09.350	Diethyl fumarate	3900	Class I	1800
09.351	Diethyl maleate	3900	Class I	1800
09.352	Diethyl nonanedioate	3900	Class I	1800
09.353	Diethyl oxalate	3900	Class I	1800
09.354	Diethyl pentanedioate	3900	Class I	1800
09.360	Ethyl 2-acetoxypropionate	3900	Class I	1800
09.502	Ethyl butyryl lactate	3900	Class I	1800
09.558	Dimethyl malonate	3900	Class I	1800
09.565	Hex-3-enyl 2-oxopropionate	3900	Class I	1800
09.580	Hexyl lactate	3900	Class I	1800
09.590	Isobutyl lactate	3900	Class I	1800
09.601	Isopentyl lactate	5100	Class I	1800
09.626	Methyl 2-oxopropionate	3900	Class I	1800
09.629	Methyl 3-acetoxyhexanoate	3900	Class I	1800
09.633	Methyl 5-hydroxydecanoate	3900	Class I	1800
09.634	Methyl acetoacetate	3900	Class I	1800
09.644	Methyl lactate	3600	Class I	1800
09.683	Pentyl lactate	3900	Class I	1800
09.815	Propyl lactate	3900	Class I	1800
09.832	Ethyl 3-acetohexanoate	3900	Class I	1800
09.833	iso-Propyl 4-oxopentanoate	3900	Class I	1800
09.862	Ethyl 3-acetoxy octanoate	3900	Class I	1800
09.874	Di(2-methylbutyl) malate	3900	Class I	1800
09.916	Ethyl 3-hydroxyoctanoate	3900	Class I	1800
10.038	Dec-7-eno-1,4-lactone	3900	Class I	1800
10.039	cis-Dec-7-eno-1,4-lactone	3900	Class I	1800
10.040	Dec-8-eno-1,5-lactone	3900	Class I	1800
10.045	Heptano-1,5-lactone	3900	Class I	1800
10.047	Hexadecano-1,16-lactone	3900	Class I	1800
10.048	Hexadecano-1,4-lactone	3900	Class I	1800
10.049	Hexadecano-1,5-lactone	3900	Class I	1800
10.052	3-Methylnonano-1,4-lactone	3900	Class I	1800
10.055	Pentano-1,5-lactone	3900	Class I	1800
10.058	Tridecano-1,5-lactone	3900	Class I	1800
10.059	Hexadec-7-en-1,16-lactone	3900	Class I	1800
10.063	Hexadec-9-en-1,16 lactone	3900	Class I	1800
10.068	Pentadecano-1,14-lactone	3900	Class I	1800
10.168	5,6-Dimethyl-tetrahydro-pyran-2-one	3900	Class I	1800
09.824	Ethyl 2-acetylbutyrate	3900	Class I	1800
06.088	2-Ethyl-4-methyl-1,3-dioxolane	3900	Class II	540
06.090	4-Hydroxymethyl-2-methyl-1,3-dioxolane	3900	Class II	540
06.095	4-Methyl-2-propyl-1,3-dioxolane	3800	Class II	540
06.135	2-Isobutyl-4-methyl-1,3-dioxolane		Class II	540
02.242	2-Butoxyethan-1-ol	3900	Class II	540
06.097	1,1,3-Triethoxypropane	3900	Class II	540
06.102	2-Hexyl-5-hydroxy-1,3-dioxane	4100	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

III.1.1. Equilibrium Between Aliphatic Lactones and Ring-opened Hydroxycarboxylic Acids: Effect of pH

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured while in acidic media, such as gastric juice and urine, the lactone ring is favoured (see Figure III.1). Enzymes, such as lactonase, may catalyse the hydrolysis reaction, but for simple saturated lactones, the ring-opening reaction and reverse cyclisation are in equilibrium, mainly controlled by pH conditions. Both the aliphatic lactones and the ring-opened hydroxycarboxylic acids can be absorbed from the gastrointestinal tract. However, the simple lactones with low molecular weight being uncharged may cross the cell membrane more easily than the acidic form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

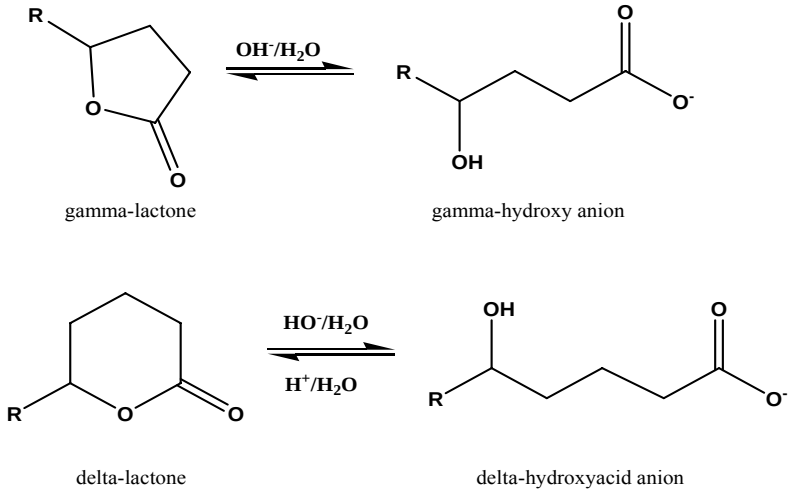


Figure III.1. *Equilibrium of gamma- and delta-lactone and hydroxycarboxylate anion*

III.1.2. Hydrolysis of Aliphatic Lactones

Fourteen candidate substances [FL-no: 10.038, 10.039, 10.040, 10.045, 10.047, 10.048, 10.049, 10.052, 10.055, 10.058, 10.059, 10.063, 10.068 and 10.168] are simple aliphatic lactones that are expected to readily undergo hydrolysis *in vivo*.

Information on the disposition of these substances is mainly derived from studies on a single supporting substance butyro-1,4-lactone [FL-no: 10.006], which has been extensively studied due to the production of CNS depression, attributed to its hydrolysis product, gamma-hydroxybutyrate. No data on the candidate substances are available.

When 4-hydroxybutanoic acid gamma-lactone (butyro-1,4-lactone) is administered intravenously (Roth and Giarman, 1966), intraperitoneally (i.p.) or orally (Guidotti and Ballotti, 1970) to rats, the open-chain 4-hydroxybutanoate anion is detected in the blood and tissues and the sedative effect produced by 4-hydroxybutanoate was evidenced (Roth and Giarman, 1966; Guidotti and Ballotti, 1970). The half-life for the conversion of the lactone ring to the open-chain anion in the blood is less than one minute. The reaction is catalysed by gamma-lactonase, which shows greater activity in the plasma than in the liver or brain (Fishbein and Bessman, 1966).

Hydrolysis of various aliphatic lactones (1 mM), including those formed from tertiary alcohols, has been described after *in vitro* incubation in basic simulated intestinal fluid and rat liver homogenate, (Morgareidge, 1962a; Morgareidge, 1963a).

Table III.1. Hydrolysis of various aliphatic lactones

Substance	Test System	% Hydrolysis	Time (hr)	Reference
Gamma-Valerolactone	Simulated intestinal fluid	32	4	(Morgareidge, 1962a)
	Rat liver homogenate	93	1	(Morgareidge, 1963a)
Gamma-Nonalactone	Rat liver homogenate (pH= 7.5)	62-94	1	(Morgareidge, 1963a)
	Rat liver homogenate (pH =8)	81-88	1	(Morgareidge, 1963a)
Gamma-Undecalactone	Simulated intestinal fluid	58	1	(Morgareidge, 1962a)
	Rat liver homogenate (pH= 7.5)	26-40	4	(Morgareidge, 1963a)
	Rat liver homogenate (pH= 8)	45-70	1	(Morgareidge, 1963a)
Omega-6-Hexadecenlactone	Simulated intestinal fluid	92	0.25	(Morgareidge, 1962a)
	Simulated intestinal fluid	96	1	(Morgareidge, 1963a)
4,4-Dibutyl-gamma-butyrolactone	Simulated intestinal fluid	92	1	(Morgareidge, 1962a)

As shown in Table III.1, the rate and the extent of hydrolysis differ, depending on the lactone tested. The observation that gamma-lactones, sterically hindered gamma-lactones, and omega-lactones are hydrolysed to the ring-opened form under these conditions supports the conclusion that the ring-opened hydroxycarboxylic acid anion exists in body fluids at basic pH. In acidic media, such as the gastric juice and the urine, the lactone form predominates.

Gamma-valerolactone and gamma-hexalactone have been detected in the urine of normal human adults (Zlatkis and Liebich, 1971).

III.1.3. Absorption of Aliphatic Lactones

Aliphatic lactones or the ring-opened hydroxycarboxylic acids are expected to be absorbed from the gastrointestinal tract. In rats, single oral doses >100 mg/kg bw/day of the supporting substance gamma-butyrolactone [FL-no: 10.006] were absorbed rapidly and completely from the intestinal tract (Arena and Fung, 1980; Guidotti and Ballotti, 1970; Lettieri and Fung, 1978). However, the lactone being an uncharged low molecular weight molecule may cross the cell membrane more easily than the ring-opened form, which penetrates the cells as a weak electrolyte (Guidotti and Ballotti, 1970).

In humans, paraoxonase (PON1), a serum enzyme belonging to the class of A-carboxyesterases (Aldridge, 1953), is known to rapidly hydrolyse a broad range of aliphatic lactone substrates including beta-, gamma-, delta- and omega-lactones, lactones fused to alicyclic rings such as 2-(2-hydroxycyclopent-4-enyl)ethanoic

acid gamma-lactone (Billecke et al., 2000). Activities of paraoxonase isoenzymes (Q & R) in human blood exhibit a bimodal distribution that is accounted for by a Q/R (glutamine or arginine) polymorphism with Q-type homozygotes showing a lower activity than QR heterozygotes or R homozygotes (Humbert et al., 1993).

Incubation of 1 mM of human R-type PON1 with aliphatic lactones gamma-butyrolactone, gamma-valerolactone, gamma-decanolactone and undecano-gamma-lactone resulted in hydrolysis rates of 9.1, 7.0, 19.0 and 13.0 µmol/min/ml substrate, respectively (Billecke et al., 2000). Hydrolysis is slower for the alicyclic fused-ring lactone, 2-(2-hydroxycyclopent-4-enyl)ethanoic acid gamma-lactone, with a hydrolysis rate of less than 3 µmol/min/ml substrate in the Q and R isoenzymes of PON1 (Billecke et al., 2000).

Based on these data, it is concluded that a wide variety of lactones readily hydrolyse in human blood serum support either prior to absorption or upon entering systemic circulation.

III.1.4. Metabolism of Lactones Formed From Linear and Branched-chain Aliphatic Hydroxy-carboxylic Acids

No literature data on the candidate substances are available; however, due to the simple structure of the substances, information on their metabolic fate may be derived from text books.

Linear aliphatic hydroxycarboxylic acids are hydrolysed and rapidly oxidised *via* the fatty acid pathway. Linear saturated 5-hydroxycarboxylic acids formed from delta-lactones are converted, *via* acetyl coenzyme A (CoA), to hydroxythioesters, which then undergo beta-oxidation and cleavage to yield an acetyl CoA fragment and a new beta-hydroxythioester reduced by two carbons. Even numbered-carbon acids continue to be oxidised and cleaved to yield acetyl CoA while odd numbered-carbon acids yield acetyl CoA and propionyl CoA. Acetyl CoA enters the citric acid cycle directly while propionyl CoA is transformed into succinyl CoA, which then enters the citric acid cycle (Voet and Voet, 1990).

Linear saturated 4- or 6-hydroxycarboxylic acids formed from gamma- or epsilon-lactones participate in the same pathway as linear saturated 5-hydroxycarboxylic acids; however, loss of an acetyl CoA fragment produces an alpha-hydroxythioester, which undergoes oxidation and alpha- decarboxylation to yield a linear carboxylic acid and eventually carbon dioxide (Voet and Voet, 1990). In rats and dogs, the supporting substances, $^{14}CO_1$-gamma-decalactone and $^{14}CO_1$-gamma- dodecalactone, are metabolised in a manner similar to $^{14}CO_1$-lauric acid, with approximately 75 % of the labeled ^{14}CO being eliminated as carbon dioxide within 48 hours (Fassett, 1961).

The metabolic fate of the supporting substance butyro-1,4-lactone [FL-no: 10.006] has been extensively studied in animals and humans. The majority of ^{14}C-labeled 4-hydroxybutanoate administered by intravenous injection to rats was recovered as $^{14}CO_2$ within 2.5 hours (Roth and Giarman, 1965). Oxidation of gamma-butyrolactone to succinate by alcohol dehydrogenase and succinic semialdehyde dehydrogenase occurs primarily in the liver (Jakoby and Scott, 1959); succinate then participates in the citric acid cycle (Doherty and Roth, 1978; Lee, 1977; Möhler et al., 1976; Walkenstein et al., 1964). However, this pathway accounts for only a limited proportion of the metabolised compound. The main biotransformation route through which gamma-butyrolactone is metabolised is beta-oxidation as indicated by the presence of (S)-3,4-dihydroxybutyric acid, glycolic acid and 3-oxobutyric acid in the urine of human volunteers given orally 1.0 g gamma-butyrolactone [FL-no: 10.006] (Lee, 1977); other intermediates derived from beta-oxidation have been previously detected in samples of human urine (Walkenstein et al., 1964).

If the lactone is formed from a linear hydroxycarboxylic acid containing unsaturation, cleavage of acetyl CoA units will continue along the carbon chain until the position of unsaturation is reached. If the unsaturation begins at an odd-numbered carbon, acetyl CoA fragmentation will eventually yield a 3-enoyl CoA, which is converted to the *trans*- Δ_2-enoyl CoA before entering the fatty acid pathway. If unsaturation

begins at an even-numbered carbon, acetyl CoA fragmentation yields a Δ_2-enoyl CoA product, which is a substrate for further fatty acid oxidation. If the stereochemistry of the double bond is *cis*, hydration yields (R)-3-hydroxyacyl CoA, which is isomerised to (S)-3-hydroxyacyl CoA by 3-hydroxyacyl CoA epimerase prior to entering into normal fatty acid metabolism (Voet and Voet, 1990).

The principal metabolic pathways utilised for detoxication of branched-chain hydroxycarboxylic acids are influenced by the chain length and the position and size of alkyl substituents. Short-chain (< C6) branched aliphatic hydroxycarboxylic acids may be excreted conjugated mainly with glucuronic acid, or undergo alpha- or beta-oxidation followed by cleavage and complete metabolism to CO_2 (Voet and Voet, 1990; Williams, 1959a) via the fatty acid pathway and the tricarboxylic acid cycle. Alternatively, as chain length, substitution and lipophilicity increase, the hydroxycarboxylic acid may undergo a combination of omega-, omega-1 and beta-oxidation to yield polar hydroxyacid, ketoacid and hydroxydiacid metabolites that may be excreted as the glucuronic acid or sulphate conjugates in the urine and, to a lesser extent, in the faeces. Methyl substituted carboxylic acids are, to some extent, omega-oxidised in animals to form diacids, which can be detected in the urine (Williams, 1959a).

Carboxylic acids with a methyl substituent located at an even-numbered carbon (e.g. 2-methylpentanoic acid or 4-methyldecanoic acid) are metabolised extensively in the fatty acid pathway to CO_2 via beta-oxidation and cleavage of the longer branched-chain. If the methyl group is located at an odd-numbered carbon such as the 3-position, beta-oxidation is inhibited and omega-oxidation predominates, primarily leading to polar, acidic metabolites capable of being excreted in the urine as such or as conjugates (Williams, 1959a). Larger alkyl substituents (>C2) located at the alpha- or beta-position inhibit metabolism to CO_2 (Deisinger et al., 1994; Deuel, 1957; Albro, 1975) in which case there is either direct conjugation of the acid with glucuronic acid or omega-oxidation leading to diacid metabolites, which may be conjugated and excreted.

III.2. Absorption, Metabolism and Elimination of: Esters, Acetals, Aliphatic Primary Alcohols, Aldehydes and Carboxylic Acids Containing Additional Oxygenated Functional Groups

III.2.1. Mono- and Di-esters

Thirty-one candidate substances are esters or diesters [FL-no: 09.333, 09.345 - 09.354, 09.360, 09.502, 09.558, 09.565, 09.580, 09.590, 09.601, 09.626, 09.629, 09.633, 09.634, 09.644, 09.683, 09.815, 09.824, 09.832, 09.833, 09.862, 09.874 and 09.916]. They are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy or keto alcohols) and acid components (i.e. alpha-, beta- or gamma-keto or hydroxy acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised. The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters; therefore the discussion and conclusions presented in previous evaluations (FGE.01 and FGE.02) apply equally well to the candidate esters in the present evaluation.

Hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases or esterases (Heymann, 1980), the most important of which are the B-esterases (Anders, 1989; Heymann, 1980). Acetyl esters are the preferred substrates of C-esterases (Heymann, 1980). In mammals, these enzymes occur in most tissues throughout the body (Anders, 1989; Heymann, 1980) but predominate in the hepatocytes (Heymann, 1980).

The majority of degradation products yielded from the candicate ester hydrolysis are endogenous in mammals and are known to be completely metabolised, through different reactions, depending on their chain length and degree of branching and functional groups. It is likely that multiple metabolic reactions will occur for some hydrolysis products. The most probable metabolic reactions are the following:

- Oxidation of alcohols to aldehydes and acids.
- Conjugation of alcohols and acids to glucuronides and sulphates.
- Beta-oxidation of carboxylic acids.
- Omega-oxidations of carboxylic acids.

However, the hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, has some structural similarities to valproic acid, which together with a number of its derivatives has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Although it can be predicted that 2-acetyl butyric acid is further metabolised through the above mentioned pathways of detoxication for carboxylic acids, the structural similarity with valproic acid does no allow to anticipate that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocuous products.

While no hydrolysis data have been provided for the esters of the present group of flavourings, information on some structurally related esters could be found.

In vitro incubation of the supporting substance methyl 2-oxo-3-methylvalerate [FL-no: 09.550], with a 2 % pancreatin solution (pH = 7.5) resulted in virtually complete hydrolysis (> 98 %) within 80 minutes (Leegwater and VanStraten, 1979). The supporting substance dibutyl sebacate [FL-no: 09.474] in 10 % acacia solution, was hydrolysed *in vitro* in a 10 % crude pancreatic lipase solution (Smith, 1953b).

The supporting substance, ^{14}C-tributyl acetylcitrate [FL-no: 09.511], administered to male Sprague-Dawley rats by gavage at a dose level of 70 mg/kg bw was rapidly absorbed ($t_{½}$ = 1 hour) and partially hydrolysed. Greater than 87 % of the administered radioactivity was eliminated within 24 hours after dosing. At least nine urinary metabolites (59 - 70 %) were detected. Five were positively identified as the partially hydrolysed mono-, di- and tri-alkylesters of citric acid. Three metabolites (25-26 %) were identified in the faeces; approximately 2 % of the administered dose was eliminated as $^{14}CO_2$ (Hiser et al., 1992).

III.2.2. Acetals

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135] are acetals, which may undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption.

In vitro experiments using simulated gastric fluid revealed the rates of hydrolysis of acetals to be dependent on the structures of the aldehyde and alcohol moieties. Acetals derived from short (< C8) chain saturated aldehydes were hydrolysed almost instantly (K-H Engel, 2003).

Hydroxycitronellal dimethyl acetal similar to the supporting substance hydroxycitronellal diethyl acetal was > 99 % hydrolysed *in vitro* to the terpenoid hydroxycitronellal and ethanol in simulated gastric juice (pH about 2.1) after 1 hour and > 6 % hydrolysed in intestinal fluid (pH = 7.5) after 2 hours (Morgareidge, 1962b).

Once hydrolysed, the component alcohol, aldehydes and acids are expected to be completely metabolised, through the above mentioned common routes of biotransformations and excreted.

III.2.3. Alpha-hydroxy- and Alpha-keto- acids and Their Esters

One candidate substance [FL-no: 08.090] is an alpha-hydroxyacid. In addition alpha-keto- and alpha-hydroxyacids are formed by hydrolysis of candidate esters [FL- No: 09.333, 09.346, 09.353, 09.565, 09.580,

09.590, 09.601, 09.626, 09.644, 09.683, 09.815 and 09.874]. They would be expected to be metabolised like endogenous alpha-ketoacids formed from oxidative deamination of amino acids such as isoleucine, methionine and valine *in vivo*.

The supporting substance, 2-oxobutyric acid [FL-no: 08.066] (i.e. alpha-ketobutyric acid), is endogenous in humans as a product of methionine degradation and undergoes alpha-decarboxylation to yield propionyl CoA. Propionyl CoA ultimately enters the tricarboxylic acid cycle as succinyl CoA (Voet and Voet, 1990).

III.2.4. Beta-keto- and Beta-hydroxyacids and Their Esters

One candidate substance [FL-no: 08.053] is a beta-ketoacid. In addition seven candidate substances [FL-no: 09.346, 09.558, 09.634, 09.824, 09.862, 09.874 and 09.916] are precursor of acetoacetic acid or its beta-hydroxy or aldehyde precursor. The latter two can be oxidised *in vivo* to acetoacetic acid. Acetoacetic acid is endogenous in humans and is formed from the condensation of two acetyl CoA units in the fatty acid pathway. It is released from the liver into the bloodstream and transported to peripheral tissues where it is converted to acetyl CoA and is completely metabolised. At elevated endogenous levels, beta-ketoacids may undergo non-enzymatic decarboxylation, which, for acetoacetic acid, yields acetone and CO_2 (Voet and Voet, 1990).

III.2.5. Gamma-keto- and Gamma-hydroxyacids and Their Esters

Gamma-hydroxy and gamma-keto acids are produced by hydrolysis of two candidate substances [FL-no: 09.832 and 09.833]. They are expected to be completely metabolised to CO_2 at low levels of exposure from use as flavouring substances. At elevated levels of exposure, the ketone function may be reduced to the corresponding secondary alcohol (Bosron and Li, 1980) and excreted as the glucuronic acid conjugate (Williams, 1959a).

Products of partial beta-oxidation or glucuronic acid conjugation have been also identified in the urine. When 1.0 g of the structurally related substance gamma-hydroxybutyrate [FL-no: 10.006] was administered to humans, it was excreted in the urine as *S*-3,4-dihydroxybutyrate, 3-oxobutyric acid and glycolate (Lee, 1977).

III.2.6. Aliphatic Di- and Tricarboxylic Acids and Their Esters

Among candidate substances the aliphatic di- and tri-carboxylic acids and their precursors [FL-no: 05.149, 08.082, 08.053, 08.103, 08.113, 09.345, 09.346, 09.347, 09.348, 09.349, 09.350, 09.351, 09.352, 09.353, 09.354, 09.558 and 09.874] either occur endogenously in humans or are structurally related to endogenous substances. Succinic acid (from [FL-no: 09.345 and 09.347]), fumaric acid (from [FL-no: 09.350]), *l*-malic acid (from [FL-no: 09.346 and 09.874]), maleic acid (from [FL-no 09.351]) and citric acid (from [FL-no: 09.349]), are components of the tricarboxylic acid cycle (Voet and Voet, 1990). Fumaric acid is present in the blood, brain, liver, muscle and kidney of normal rats (Marshall et al., 1949). Moreover, the following acids are present in the urine of normal adults, citric, tartaric, malic, aconitic, fumaric and adipic (Hanson, 1943; Osteux and Laturaze, 1954). Alpha-ketoglutaric acid is an intermediate metabolite of citric acid, fumaric acid and succinic acid, and is formed via alpha-oxidation (Krebs et al., 1938; Simola and Krusius, 1938).

Simple aliphatic di- and tricarboxylic acid candidate substances and component acids of the candidate esters are metabolised in the fatty acid beta-oxidation pathway or tricarboxylic acid cycle. When the supporting substance ^{14}C-*l*-malic acid [FL-no: 08.017] was administered to male albino Wistar rats by gavage at a dose level of 2.5 mg/kg bw, 93 % of the radioactivity was recovered in expired air, urine and faeces (Dargel, 1966).

After the administration of the radioactive supporting substance adipic acid [FL-no: 08.026] to rats by stomach tube at a dose level of 200-300 mg/kg bw, the compound was extensively metabolised. Labelled products identified in the urine included glutamic acid, lactic acid, beta-ketoadipic acid and citric acid. The presence of the beta-oxidation metabolite, beta-ketoadipic acid, indicates that adipic acid participates in beta-oxidation in the fatty acid pathway (Rusoff et al., 1960).

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids (Bosron and Li, 1980; Feldman and Weiner, 1972; Levi and Hodgson, 1989). The resulting carboxylic acids would be metabolised in the fatty acid pathway and tricarboxylic acid cycle (Voet and Voet, 1990) or conjugated to glucuronides and sulphates and excreted. Branched-chain diols or keto alcohols may undergo oxidation to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted, through the common routes of biotransformation of carboxylic acids.

III.2.7. Aliphatic Alkoxy- alcohol and Diols

Among candidate substances, one is an alkoxy-alcohol [FL-no: 02.242] and two are diols [FL-no: 02.132 and 02.198].

The metabolism and disposition of 2-butoxyethanol [FL-no: 02.242] were extensively studied, and details are reported below. However, it can be anticipated that the major metabolite is butoxyacetic acid, which is primarily responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

1-Hydroxypropan-2-one [FL-no: 07.169] (acetol) is an endogenous metabolite of acetone which is also an endogenous substance formed from the degradation of body fat/fatty acids.

The metabolism in mammals of acetone, which at low concentrations, primarily occurs in the liver, is shown in Figure III.2. At low acetone concentrations in blood, i.e. in healthy humans not exposed to external sources in amounts of approximately 4-12 mg per person corresponding to 0.7 to 2 mg/l blood (Ashley et al., 1994; Dick el al., 1988; Wang et al, 1994c), the major pathway is via the methylglyoxal route. At higher acetone concentrations in the blood e.g. after acetone exposure, after fasting or in relation to certain deseases, the propan-1,2-diol route is the dominating pathway.

In the fist step acetone is oxidized to 1-hydroxypropan-1,2-one via acetone monooxygenase (p-450 IIE1). 1-Hydroxypropan-2-one is oxidised to 2-oxopropanal via acetol monooxygenase (p-450 IIE1), or at higher acetone concentrations to propan-1,2-diol. 2-Oxopropanal is then oxidised to pyruvate leading to glucose formation (Morgott, 1993; WHO, 1998a; NAS/COT, 2005).

The diols are anticipated to be metabolised by the common route of alcohol biotransformation, i.e. direct conjugation or oxidation by alcohol-dehydrogenase to their corresponding aldehydes and carboxylic acid, which would be further metabolised or excreted.

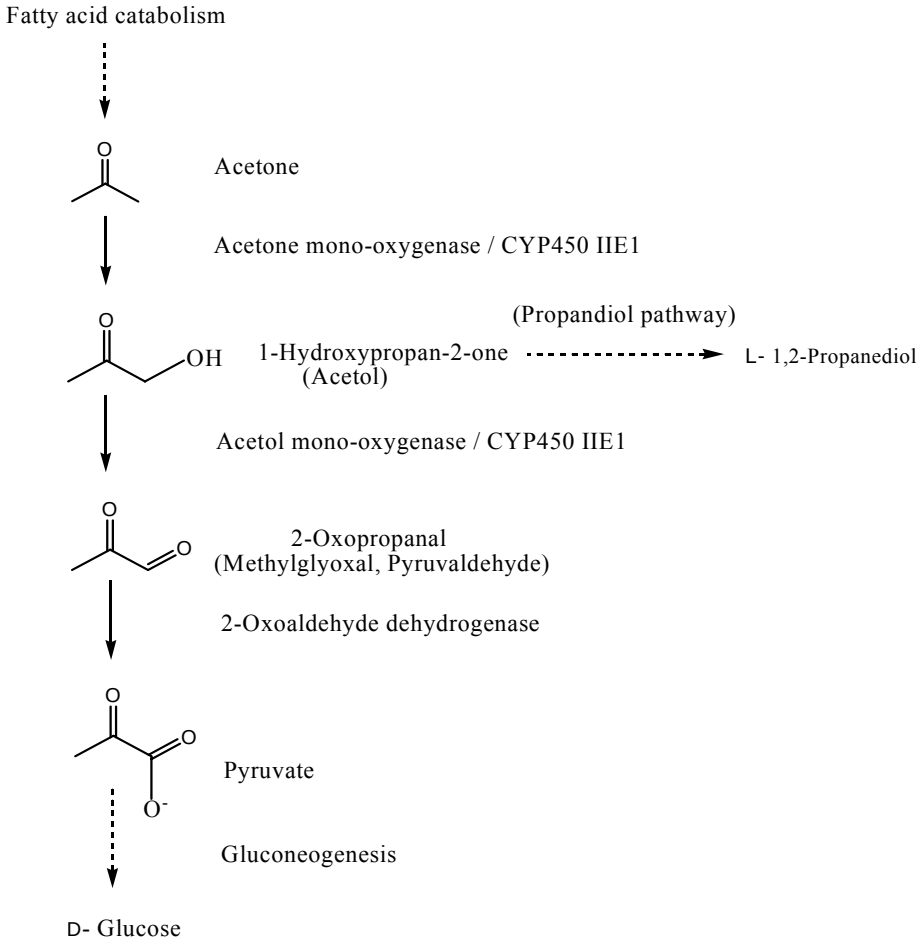


Figure III.2. *Acetone metabolism (methylglyoxal pathway)*

III.3. Studies on Candidate Substances

2-Butoxyethan-1-ol [FL-no: 02.242]

Several experiments by the oral route of administration have been conducted that indicate 2-butoxyethan-1-ol is rapidly absorbed, metabolised and eliminated. Butoxyacetic acid is its major metabolite, metabolism being mainly catalysed by hepatic alcohol dehydrogenase; most excretion is in the urine (Corley et al., 1994; Ghanayem et al., 1987a; Ghanayem et al., 1987b; Ghanayem et al., 1987c; Medinsky et al., 1990).

The distribution and excretion of ^{14}C-butoxyethanol and its metabolites was evaluated using male F344 rats (9 -13 weeks old). A single 125 or 500 mg/kg dose of ^{14}C-butoxyethanol was administered to each animal via gavage. Animals were killed 48 hours post-administration and tissues excised. At 48 hours, approximately 18 % and 10 % of the administered dose was exhaled as $^{14}CO_2$ for the 125 and 500 mg/kg doses, respectively; whereas only between 2 and 3 % was excreted in the faeces. The percentage of the 125 mg/kg dose excreted in the urine (70 %) was significantly greater than the percentage excreted after the 500 mg/kg dose (40 %). Butoxyacetic acid was the only urinary metabolite detected for the 125 mg/kg dose; the glucuronide conjugates of butoxyethanol and butoxyacetic acid (23 %) were also detected in the urine of animals dosed with the higher dose. A small portion (8 %) of the 500 mg/kg dose was excreted in the bile in the 8 hours after dosing. Compared to the 125 mg/kg dose group, tissue concentrations of ^{14}C-butoxyethanol

48 hours after administration were significantly greater in specific organs of rats that received the 500 mg/kg dose. In both dose groups the highest concentration of radioactivity was detected in the forestomach, followed by the liver, kidneys, spleen and the glandular stomach (Ghanayem et al., 1987c).

The metabolism and excretion of 2-butoxyethan-1-ol [FL-no: 02.242] were evaluated using both young (4 to 5 weeks old) and adult (9 to 13 weeks old) male F344 rats with the same experimental design described in Ghanayem *et al*. (1987c), except that ^{14}C-butoxyethanol was administered at a single oral dose (500 mg/kg). There was a significantly higher proportion of the administered dose eliminated as CO_2 in young rats as compared to older rats. Similarly, a significantly higher proportion of the administered dose was excreted in the urine of the young rats. The butoxyacetic acid/butoxyethanol-glucuronide + butoxyethanol-sulphate ratio was significantly greater in older rats (Ghanayem et al., 1987a), which are consistently more susceptible to the toxic action of 2-butoxyethan-1-ol . There was a strong correlation between the amount of butoxyacetic acid in the urine and 2-butoxyethanol-induced haematotoxicity. Moreover, metabolic activation via alcohol and aldehyde dehydrogenases ia a prerequisite for the induction of toxic effects, since pre-treatment of rats with pyrazole (alcohol dehydrogenase inhibitor) or cyanamide (aldehyde dehydrogenase inhibitor) protected rats against 2-butoxyethanol-induced haematotoxicity and increased the urinary amount of butoxyethanol-conjugates (glucuronide and silphate) (Ghanayem et al., 1987b).

2-Butoxyethan-1-ol [FL-no: 02.242] was administered to male F344/N rats (11 to 12 weeks old) at concentrations in drinking water of 290, 860 and 2590 ppm over a 24 hour period. Butoxyethanol was administered as 2-butoxy[U-^{14}C]ethanol, and exhaled air, urine and faeces were collected over a 72 hours period. Most ^{14}C was excreted either in the urine or exhaled as CO_2: 50-60 % of the administered dose was eliminated in the urine as butoxyacetic acid and 8 to 10 % as CO_2. Analysis of urine samples collected during the 12 - 24 hours after dosing indicated that the majority of the radioactivity was associated with butoxyacetic acid while 10 % of the administered dose was identified as glycol ether. Minor levels of glucuronide conjugate of butoxyethanol and unmetabolised butoxyethanol were also reported (Medinsky et al., 1990).

Non-oxidative metabolism of 2-butoxyethan-1-ol [FL-no: 02.242] via fatty acid conjugation was also investigated in the liver of F344 male rats following a single oral administration of 500 mg/kg [ethyl-1,2-^{14}C] 2-butoxyethanol. Animals were killed two hours after treatment and samples prepared for analysis. It was demonstrated that 2-butoxyethan-1-ol is metabolised non-oxidatively via conjugation with long-chain fatty acids, and the formation of these esters appears to be catalysed by the enzymes involved in fatty acid conjugation of xenobiotic alcohols. However, the biological significance of 2-butoxyethan-1-ol conjugation with fatty acids remains unclear, although several such lipid conjugates were found to be toxic in laboratory animals and cell lines (Kaphalia et al., 1996).

The elimination kinetics of 2-butoxyethan-1-ol were studied in a once-through isolated perfused rat liver system in the presence and absence of ethanol. Dose-dependent Michaelis-Menten kinetics were observed in the elimination of 2-butoxyethan-1-ol. The apparent K_m ranged from 0.32 to 0.70 mM and the maximum elimination rate ranged from 0.63 to 1.4 micromol/min/g liver in six experiments. The results support the hypothesis that 2-butoxyethan-1-ol is metabolised mainly via oxidation by alcohol dehydrogenase in the rat liver at concentration which can considered representative of human exposure (Johanson et al., 1986).

Butane-1,3-diol [FL-no: 02.132]

Two groups of 14 rats were administered a control diet (70 % carbohydrate and 30 % fat) or a treatment diet (45 % carbohydrate, 30 % fat and 25 % butane-1,3-diol). Blood acetoacetate and beta-hydroxybutyrate concentrations were increased significantly and blood pyruvate concentration was decreased significantly in rats administered the treatment diet. Addition of butane-1,3-diol to *in vitro* liver tissue slices as they were metabolising glucose to lactate and pyruvate, greatly decreased pyruvate levels and significantly increased lactate/pyruvate ratios. When butane-1,3-diol and glucose were used as substrates, there was a large increase in acetoacetate and beta-hydroxybutyrate formation in liver tissue slices with butane-1,3-diol. Therefore,

butane-1,3-diol is metabolised in the cytosol and converted by the liver *in vivo* and *in vitro* to ketones prior to its oxidation in the tricarboxylic acid cycle (Mehlman et al., 1971).

Tate *et al.* (1971) found that the conversion of butane-1,3-diol to beta-hydroxybutyrate in rat liver was strongly dependent in NAD^+ and it was inhibited by pyrazole. Since pyrazole is a specific inhibitor of alcohol dehydrogenase (ADH), this inhibition indicated ADH as the catalyst in the catabolism in the cytosol of butane-1,3-diol to an intermediate, aldol. Aldol is then further oxidised to beta-hydroxybutyrate (Tate et al., 1971).

Diethyl maleate [FL-no: 09.351]

Traditionally diethyl maleate [FL-no: 09.351] has been utilised to acutely deplete reduced glutathione (GSH) in the tissues, since it forms GHS-conjugates very rapidly causing a significant decrease in GSH content (Boyland & Chasseaud, 1970). The liver is the most sensitive organ to diethyl maleate-induced GSH depletion, generally occurring 30-90 minutes after intraperitoneal injection of the compound. In the rat, the formed GSH-conjugates are excreted in bile or as mercapturates in urine (Barnhart and Combes, 1978).

The excretion of mercapturic acid was determined in chimpanzees and rats after the administration of diethyl maleate [FL-no: 09.351]. The excretion rate of endogenous thioethers in the urine of untreated chimpanzees and rats was 18.0 and 94.4 micromol/kg bw/24 hours, respectively. The value in man was nearly the same as found in chimpanzees. The administration of diethyl maleate at 30, 75 and 200 mg/kg bw led to a dose-dependent increase in the excretion of urinary mercaptic acids in both species, but the increase in rats was about twice that of chimpanzees. Additional experiments indicate that the observed species differences are due to differences in the glutathione conjugation (Summer et al., 1979a).

Glutaric acid [FL-no: 08.082]

Rat liver mitochondria metabolise glutarate [FL-no: 08.082] at a slow rate as compared with glutaryl CoA. The stimulatory effect of citric acid cycle intermediates, NAD and CoA on glutarate metabolism was interpreted as a manifestation of their involvement in the activation of glutarate by a thiol transferase with succinyl CoA as the coenzyme A donor (Besrat et al., 1969).

Glutaraldehyde [FL-no: 05.149]

Material mass balance and pharmacokinetics studies were conducted with glutaraldehyde [FL-no: 05.149] in groups of F344 rats (four/sex) and New Zealand white rabbits (two/sex) using the intravenous route of exposure at dose volumes of 0.2 ml and 2.5 ml, respectively. Rats and rabbits received intravenous doses of 0.075 and 0.75 % glutaraldehyde in the tail vein or ear vein, respectively. Glutaraldehyde was distributed rapidly and eliminated when administered intravenously to rats and rabbits. When a single infusion of 0.075 % glutaraldehyde was administered, 75 to 80 % of the dose in the rat and 66 to 71 % in the rabbit were recovered as $^{14}CO_2$ during the first 24 hours following administration, with 80 % of the $^{14}CO_2$ being recovered during the first four hours. When a single infusion of 0.75 % glutaraldehyde was administered, the proportion of the dose recovered as $^{14}CO_2$ decreased and the amount of radioactivity recovered in urine, tissues and carcass increased as compared to the 0.075 % glutaraldehyde infusion. Also, the average plasma concentration of radioactivity increased 10-fold in rats and rabbits with a 10-fold increase in dose, but the tissue concentration increased by an even greater amount. The results suggest that the mechanisms involved in the disposition of glutaraldehyde were saturated when the higher dose was administered and resulted in a shift in the elimination pathway (McKelvey et al., 1992). Although the metabolism of glutaraldehyde has not been studied in detail, it has been suggested that it is oxidised first to a mono- or dicarboxylic acid by aldehyde dehydrogenase (Weiner, 1980; Hjelle and Petersen, 1983) and then further oxidised through an acidic intermediate to CO_2 (McKelvey et al., 1992).

Nonanedioic acid [FL-no: 08.103]

Following intravenous administration in human volunteers, nonanedioic acid [FL-no: 08.103] and its major catabolite, pimelic acid, are found in serum and urine indicating transformation by mitochondrial beta-oxidative enzymes. Serum levels of nonanedioic acid are short-lived following a single 5 or 10 g intravenous (i.v.) infusion over 1-hour. In the first hour after the cessation of i.v. administration, serum levels of nonanedioic acid decreased to about 25 % of their peak values. Administration of multiple intravenous doses at the same concentrations as the one-hour doses produces sustained higher levels of nonanedioic acid in the serum during the period of administration (Passi et al., 1989).

III.4. Conclusions

In general, lactones are formed by acid-catalysed intramolecular cyclisation of hydroxycarboxylic acids. In an aqueous environment, a pH-dependent equilibrium is established between the open-chain hydroxycarboxylate anion and the lactone ring. In basic media, such as blood, the open-chain hydroxycarboxylate anion is favoured, while in acidic media, such as gastric juice and urine, the lactone ring is favoured.

Lactones formed from linear saturated and branched-chain aliphatic hydroxycarboxylic acids are hydrolysed to the corresponding hydroxycarboxylic acid that then enters the fatty acid pathway and undergoes alpha- or beta-oxidation and cleavage to form acetyl CoA and a chain-shortened carboxylic acid. The carboxylic acid is then reduced by two-carbon fragments until either acetyl CoA or propionyl CoA is produced. These fragments then are completely metabolised in the citric acid cycle.

Mono- and di-esters included in the present FGE are expected to undergo hydrolysis in humans to yield their corresponding alcohol (saturated linear or branched-chain aliphatic primary alcohols, or branched-chain hydroxy- or keto-alcohols) and acid components (i.e. alpha-, beta- or gamma-keto- or hydroxy-acids; or simple aliphatic acids, diacids or triacids), which would be further metabolised and excreted through the common pathways of detoxication of aliphatic alcohols and carboxylic acids). The hydrolysis product of the candidate substance ethyl 2-acetylbutyrate [FL-no: 09.824], 2-acetyl butyric acid, which shows some structural similarities to valproic acid, which together with a number of its derivatives, has been recognised to be teratogenic in rodents and in humans (Nau and Löscher, 1986; Samren et al., 1997; Kaneko et al., 1999). Therefore, it cannot be anticipated that ethyl 2-acetylbutyrate [FL-no: 09.824] is metabolised to innocous products.

The presence of a second oxygenated functional group has little if any effect on hydrolysis of these esters. The most probable metabolic reactions of the hydrolysis products are oxidation of alcohols to aldehydes and acids; conjugation of alcohols and acids to glucuronides and sulphates; beta-oxidation of carboxylic acids; omega-oxidations of carboxylic acids.

Beta-keto acids and derivatives like acetoacetic acid undergo decarboxylation. Along with alpha-keto and alpha-hydroxyacids, they yield breakdown products, which are incorporated into normal biochemical pathways. The gamma-keto-acids and related substances may undergo complete or partial beta-oxidation to yield metabolites that are eliminated in the urine. Omega-substituted derivatives are readily oxidised and/or excreted in the urine. Simple aliphatic di- and tricarboxylic acids participate in the tricarboxylic acid cycle.

Six candidate substances [FL-no: 06.088, 06.090, 06.095, 06.097, 06.102 and 06.135]] are acetals, which may be expected to undergo acid catalysed hydrolysis in the gastric environment to yield their component aldehydes and alcohols prior to absorption. Once hydrolysed, the component alcohols and aldehydes are expected to be metabolised primarily through the above mentioned common routes of biotransformations and excreted.

The linear and branched-chain aliphatic primary alcohol components of candidate substances that are simple aliphatic di- and tricarboxylic acid esters would be oxidised in the presence of alcohol dehydrogenase to their corresponding aldehydes which, in turn, would be oxidised to their corresponding carboxylic acids. The two diols [FL-no: 02.132 and 02.198] may be anticipated to participate in the same routes of biotransformation.

Among candidate substances, an alkoxy-alcohol 2-butoxyethanol [FL-no: 02.242] is mainly metabolised to butoxyacetic acid, which has been identified as the major responsible for the hemolysis of red blood cells and other toxic effects induced by 2-butoxyethanol.

In summary, it can be anticipated that primary and secondary aliphatic saturated or unsaturated alcohols, aldehydes, carboxylic acids, acetals and esters with an additional oxygenated functional group and aliphatic lactones included in the present FGE are generally hydrolysed and completely metabolised to innocuous products many of which are endogenous in humans, at the estimated level of intake as flavouring substances.

The consideration on the actual levels of intake becomes particularly relevant for one candidate substance, diethyl maleate [FL-no: 09.351]; as when administered at high doses, it is able to induce severe GSH depletion, due to its prompt metabolism to GSH-conjugates. This may also be the case for the structually related diethyl fumarate [FL-no: 09.350].

For three of the candidate substances it cannot be concluded that they are metabolised to innocuous products. These are 2-butoxyethanol [FL-no: 02.242], the major metabolite of which, butoxyacetic acid, has been recognised as responsible for haematotoxic effects induced by 2-butoxyethanol, 1,1,3-triethoxypropane [FL-no: 06.097] which may be metabolised to the structurally related ethoxypropanoic acid; and finally, ethyl 2-acetylbutyrate [FL-no: 09.824], whose hydrolysis gives rise to 2-acetylbutyric acid, with some structural similarities to valproic acid, a known teratogenic compound.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for 15 candidate substances of the present Flavouring Group Evaluation of 58 substances from chemical groups 9, 13 and 30 and for 49 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Methyl 2-hydroxy-4-methylpentanoate [09.548])	Mouse	NR	Oral	4000[1]	(Pellmont, 1978)
(Methyl 2-oxo-3-methylvalerate [09.550])	Rat	M	Gavage	> 5000	(Moreno, 1979b)
Isobutyl lactate [09.590]	Rat	NR	Oral	> 2000	(Riebeek, 1989)
(Butyro-1,4-lactone [10.006])	Mouse	NR	Gavage	1245	(Schafer and Bowles, 1985)
(Pentano-1,4-lactone [10.013])	Rat	NR	Oral	> 5000	(Moreno, 1978e)
	Rat	NR	Gavage	8800	(Deichmann et al., 1945)
	Rabbit	NR	Gavage	2480	(Deichmann et al., 1945)
(Hexano-1,4-lactone [10.021])	Rat	NR	Oral	> 5000	(Moreno, 1977f)
(Hexano-1,5-lactone [10.010])	Rat	M	Gavage	13,030	(Smyth et al., 1962)
(Heptano-1,4-lactone [10.020])	Rat	NR	Oral	> 5000	(Moreno, 1977g)
(Octano-1,4-lactone [10.022])	Rat	NR	Oral	> 5000	(Moreno, 1974c)
(Octano-1,5-lactone [10.015])	Rat	NR	Oral	> 5000	(Moreno, 1977h)
(Nonano-1,4-lactone [10.001])	Rat	M, F	Gavage	9780	(Jenner et al., 1964)
	Rat	M	Oral	6600	(Moreno, 1972a)
	Guinea pig	M, F	Gavage	3440	(Jenner et al., 1964)
(Decano-1,4-lactone [10.017])	Rat	NR	Oral	> 5000	(Moreno, 1975h)
(Decano-1,5-lactone [10.007])	Rat	NR	Oral	> 5000	(Levenstein, 1975c)
(Decano-1,6-lactone [10.029])	Mouse	M, F	Gavage	5252	(Moran et al., 1980)
(Undecano-1,4-lactone [10.002])	Rat	M, F	Gavage	18500	(Jenner et al., 1964)
(Undecano-1,5-lactone [10.011])	Rat	NR	Oral	> 5000	(Moreno, 1975i)
(Dodecano-1,4-lactone [10.019])	Rat	NR	Oral	> 5000	(Moreno, 1974d)
(Dodecano-1,5-lactone [10.008])	Rat	NR	Oral	> 5000	(Moreno, 1977d)
(Dodecano-1,6-lactone [10.028])	Mouse	M, F	Gavage	7898	(Moran et al., 1980)
(Pentadecano-1,15-lactone [10.004])	Rat	NR	Oral	> 5000	(Levenstein, 1974c)
(5-Methylfuran-2(3H)-one [10.012])	Mouse	M, F	Gavage	2800	(Moran et al., 1980)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat	M, F	Gavage	1600 – 5000	(Piccirillo and Hartman, 1980a)
(Mixture of 5-Hydroxy-2-decenoic acid delta-lactone, 5-Hydroxy-2-dodecenoic acid delta-lactone, and 5-Hydroxy-2-tetradecenoic acid delta–lactone)	Rat	M, F	Gavage	3363	(Reagan and Becci, 1984a)
(Dodec-6-eno-1,4-lactone [10.009])	Rat	M, F	Oral	> 5000	(Watanabe and Morimoto, 1990)
(Hexadec-6-eno-1,16-lactone [10.003])	Rat	NR	Oral	> 5000	(Wohl, 1974a)
(3,7-Dimethyloctano-1,6-lactone [10.027])	Rat	M, F	Gavage	> 5000	(Lewis and Palanker, 1979a)
(5-Hexyl-5-methyldihydrofuran-2(3H)-one [10.051])	Rat	NR	Oral	> 5000	(Moreno, 1976j)
(Citronellyl oxyacetaldehyde [05.079])	Rat	NR	Oral	> 5000	(Moreno, 1973d)
1-Hydroxypropan-2-one [07.169]	Rat	NR	Oral	2200[2]	(Smyth and Carpenter, 1948)
(4,4-Dimethoxybutan-2-one [06.038])	Rat	M	Gavage	6200	(EPA, 1971)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Ethyl acetoacetate [09.402])	Rat	NR	Oral	3980[3]	(Smyth et al., 1949)
Methyl acetoacetate [09.634]	Rat	NR	Oral	3000	(Smyth and Carpenter, 1948)
	Rat	NR	Oral	2800	(BASF, 1978)
(Butyl acetoacetate [09.403])	Rat	F	Gavage	11260	(Smyth et al., 1954)
(Geranyl acetoacetate [09.405])	Rat	NR	Oral	> 5000	(Moreno, 1976k)
(Ethyl 3-oxohexanoate [09.542])	Mouse	NR	Oral	4000 – 8000	(Pellmont, 1973a)
(Ethyl 2,4-dioxohexanoate [09.514])	Rat	M, F	Gavage	6450	(Wolven and Levenstein, 1969)
2-Butoxyethan-1-ol [02.242]	Rat	M	Gavage	1480	(Smyth et al., 1941)
	Rat	NR	Oral	1174	(BASF, 1956)
	Rat	NR	Oral	620	(Rowe and Wolf, 1982)
	Rat	M, F	Oral	2800	(Carpenter et al., 1956)
	Rat	M	Gavage	2680	(Myers and Homan, 1980)
	Rat	NR	Oral	470	(Wolf, 1959)
	Rat	M	Gavage	1190 – 2800	(Weil and Wright, 1967)
	Rat	M	Gavage	1590	(Moreno, 1976l)
	Rat	M	Gavage	7500	(Moreno, 1976l)
	Rat	NR	Oral	1746	(Eastman Kodak Co., 1989)
	Rat	M	Gavage	7292	(Eastman Kodak Co., 1984)
	Mouse	NR	Oral	1230	(Carpenter et al., 1956)
	Mouse	NR	Oral	1170 – 1700	(Dow Chemical Company, 1982a)
	Mouse	NR	Oral	1519	(Eastman Kodak Co., 1989)
	Mouse	M	Gavage	2406	(Eastman Kodak Co., 1984)
	Rabbit	M	Oral	320 – 370	(Carpenter et al., 1956)
	Guinea pig	M, F	Oral	1200	(Carpenter et al., 1956)
	Guinea pig	M, F	Gavage	1200	(Smyth et al., 1941)
Butane-1,3-diol [02.132]	Rat	F	Gavage	> 5000	(CTFA, 1978)
	Rat	M	Gavage	18610	(Smyth et al., 1941)
	Rat	M	Gavage	22800	(Smyth et al., 1951a)
	Rat	NR5	Oral	29590	(Bornmann, 1954)
	Mouse	NR5	Oral	23440	(Bornmann, 1954)
	Mouse	NR	Oral	23310	(Kopf et al., 1950; Loeser, 1949)
	Mouse	NR	Oral	12980	(Wenzel and Koff, 1956)
	Guinea pig	M, F	Gavage	11460	(Smyth et al., 1941)
(4-Oxovaleric acid [08.023])	Rat	NR	Oral	1850	(Moreno, 1977j)
(Ethyl 4-oxovalerate [09.435])	Rat	NR	Oral	> 5000	(Moreno, 1978f)
Octane-1,3-diol [02.198]	Rat	NR	Oral	> 20000	(Frankenfeld et al., 1975)
(3,7-Dimethyloctane-1,7-diol [02.047])	Rat	M, F	Gavage	> 5000	(Levenstein, 1973b)
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Rat	NR	Oral	> 5000	(Shelanski and Moldovan, 1973a)
1,1,3-Triethoxypropane [06.097]	Rat	M	Gavage	1600	(Smyth et al., 1951a)
Diethyl oxalate [09.353]	Rat	NR	Oral	400 – 1600	(Patty, 1963)
(Malonic acid [08.053])	Rat	NR	Oral	1310	(Bio-Fax, 1971)
Dimethyl malonate [09.558]	Rat	NR	Oral	4620	(Levenstein, 1976a)
	Rat	NR	Oral	5331	(Merck Index, 1992)
(Diethyl malonate [09.490])	Rat	NR	Oral	14900	(Smyth et al., 1969a)
	Mouse	NR	Gavage	5400	(Wolven and Levenstein, 1969)

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(Diethyl succinate [09.444])	Rat	NR	Oral	8530[3]	(Smyth et al., 1951a)
(Fumaric acid [08.025])	Rat	M, F	Oral	M: 10700; F: 9300	(Vernot et al., 1977)
Diethyl fumarate [09.350]	Rat	NR	Oral	1500	(Hood, 1951)
(l-Malic acid [08.017])	Rat	NR	Oral	3500	(Morgareidge, 1973a)
	Mouse	NR	Oral	2660	(Morgareidge, 1973b)
	Rabbit	NR	Oral	3000	(Morgareidge, 1973c)
Diethyl maleate [09.351]	Rat	M	Gavage	3200	(Smyth et al., 1949)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Rat	NR	Oral	7500[6]	(Foulger, 1947)
Glutaric acid [08.082]	Mouse	NR	Oral	6000	(Boyland, 1940)
Glutaraldehyde [05.149]	Rat	NR	Gavage	252	(Stonehill et al., 1963)
	Rat	M	Gavage	733[7]	(Ballantyne and Myers, 2001)
	Rat	M	Gavage	2380[8]	(Smyth et al., 1962)
	Rat	M	Gavage	540[9]	(Striegel and Carpenter, 1964)
	Rat	M, F	Oral	M: 134; F: 165	(Ikeda, 1980)
	Rat	M	Gavage	1300[7]	(Myers et al., 1977b)
	Rat	M	Gavage	1870[8]	(Myers et al., 1977c)
	Mouse	NR	Gavage	352	(Stonehill et al., 1963)
	Mouse	M, F	Oral	M: 100; F: 110	(Ikeda, 1980)
	Mouse	M, F	Gavage	M: 152[7] ; F: 113[7]	(Ballantyne and Myers, 2001)
	Mouse	M, F	Gavage	M: 151[8] ; F: 115[8]	(Union Carbide Corp., 1992)
(Adipic acid [08.026])	Mouse	M	Oral	1900[10]	(Horn et al., 1957)
Diethyl adipate [09.348]	Rat	NR	Oral	> 1600	(Patty, 1963)
Nonanedioic acid [08.103]	Rat	M, F	Gavage	> 4000	(Mingrone et al., 1983)
	Rabbit	M, F	Gavage	> 4000	(Mingrone et al., 1983)
(Diethyl sebacate [09.475])	Rat	M, F	Gavage	14470	(Jenner et al., 1964)
	Rat	M	Oral	32000[11]	(Smith, 1953a)
	Mouse	NR	Gavage	> 32000	(Lawrence et al., 1974)
(Triethyl citrate [09.512])	Rat	NR	Gavage	7000[4]	(Finkelstein and Gold, 1959)
(Tributyl acetylcitrate [09.511])	Rat	NR	Gavage	> 30000[12]	(Finkelstein and Gold, 1959)
(3-Hydroxy-2-oxopropionic acid [08.086])	Rat	NR	Oral	2000	(Hoechst, 1995)
(Succinic acid, disodium salt [08.113])	Rat	NR	Oral	>1200	MHLW Japan 2002 in: (OECD, 2003)

M = Male; F = Female

NR: Not reported

1 Dosed in 5 % gum arabic.

2 Data derived from a range-finding study.

3 Actual LD_{50} not reported.Study conducted as a dose range-finder (DRF).

4 Actual LD_{50} not reported.Value reported as approximate LD_{50}.

5 Data point not verified.

6 Actual LD_{50} not reported.Value reported as MFD (assumed to be Median Fatal Dose).

7 Glutaraldehyde dosed as a 50 % (w/w) solution.The LD_{50} is expressed as mg of actual active ingredients.

8 Test substance administered as a 25 % solution. The LD_{50} is expressed as mg of actual active ingredients.

9 Test substance administered as a 45 % aqueous solution.The LD50 is expressed as mg of actual active ingredients.

10 Dosed as a 6% suspension in 0.5 % methyl cellulose.

11 Actual LD_{50} not reported.Value represents lowest dose level tested causing mortality. Animals dosed at 16,000 mg/kg had 100 % survival rate, while animals dosed at 32,000 mg/kg had 100 % fatality. Acute lethal dose for dibutyl sebacate is between 16,000 and 32,000 mg/kg.

12 Value represents the maximum dose level tested. Animals dosed at 30,000 mg/kg had 100 % survival rate.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 and for 23 supporting substances evaluated by the JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for two structurally related substances. The supporting and structurally related substances are listed in brackets.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Mouse; M, F 5/20	Gavage	90	525	(NTP, 1992e)	a)
	Rat; M, F 5/20	Gavage	90	450	(NTP, 1992e)	a)
	Mouse; M, F 2/100	Gavage	2 years	262	(NTP, 1992e)	a)
	Rat; M, F 2/100	Gavage	2 years	112	(NTP, 1992e)	a)
	Rat; M, F 1/7	Diet	4 – 6 months	100[2]	(Fassett, 1961)	a)
(Pentano-1,4-lactone [10.013])	Rat; M, F 1/30	Diet	90	M: 49[2] ; F: 51.1[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/10	Diet	90	500[2]	(Hagan et al., 1967)	a)
(Octano-1,5-lactone [10.015])	Rat; M, F 1/7	Diet	4 - 6 months	32[2]	(Fassett, 1961)	a)
(Nonano-1,4-lactone [10.001])	Rat; M, F 1/30	Diet	90	M: 62.8[2] ; F: 72.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
	Rat; M, F 1/20	Diet	2 years	50[2]	(Bär and Griepentrog, 1967)	a)
(Decano-1,4-lactone [10.017])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
(Decano-1,5-lactone [10.007])	Rat; M, F 1/NR	Diet	49 weeks	150[2]	(Fassett, 1961)	a)
	Dog; M, F 1/NR	Diet	38 weeks	250[2]	(Fassett, 1961)	a)
(Undecano-1,4-lactone [10.002])	Rat; M, F 1/30	Diet	90	M: 14.6[2] ; F: 16.5[2]	(Oser et al., 1965)	a)
	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
	Rat; M, F 1/20	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
	Rat; M, F NR[4]	Diet	90	14.1[2, 3]	(Shillinger, 1950)	a)
(Dodecano-1,4-lactone [10.019])	Rat; M, F 1/7	Diet	4-6 months	32[2]	(Fassett, 1961)	a)
(Dodecano-1,5-lactone [10.008])	Rat; M, F 1/NR	Diet	49 weeks	300[2]	(Fassett, 1961)	a)
	Dog; M, F	Diet	38 weeks	150[2]	(Fassett, 1961)	a)

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	1/NR					
(5-Methylfuran-2(3H)-one [10.012])	Rat; M, F 1/NR	Diet	90	M: 17.4[2] ; F: 17.7[2]	(Shellenberger, 1971c)	a)
(6-Pentyl-2H-pyran-2-one [10.031])	Rat; M, F 1/30	Diet	90	12.1[2]	(Cox et al., 1974h)	a) A carefully performed one dose study not in compliance with a specific testing guideline but of sufficient quality to accept the data. "Reliable with restriction" according to (Klimisch et al., 1997).
(5-Ethyl-3-hydroxy-4-methylfuran-2(5H)-one [10.023])	Rat; M, F 1/30	Diet	90	M: 1.29[2] ; F: 1.47[2]	(Posternak et al., 1969)	a)
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Rat; M, F 6/8-16	Diet	13 - 52 weeks	46[2]	(Munday and Kirkby, 1973; Munday and Kirkby, 1971a)	a)
(Ethyl acetoacetate [09.402])	Rat; M, F 3/32	Diet	28 - 29	300	(Cook et al., 1992)	a)
2-Butoxyethan-1-ol [02.242]	Rat; M, F 4/20	Diet	91 – 93	40	(Union Carbide Corp., 1963)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	No NOAEL derived [13]	(Union Carbide Corp., 1952)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/10	Diet	90	76	(Carpenter et al., 1956)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/20	Drinking water	13 weeks	1500 ppm (150 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M 3/10	Gavage	6 weeks	222	(Krasavage, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 5/10	Drinking water	14	400	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/20	Drinking water	13 weeks	6000 ppm (1200 mg/kg/day)	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; M, F 4/6[4]	Drinking water	21	M: < 2000 ppm (200 mg/kg/day); F: < 1600 ppm (160 mg/kg/day)	(Exon et al., 1991)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F 5/10	Drinking water	14	< 150[5]	(NTP, 1993a)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M NR	Oral	5 week	1000	(Bernstein, 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/5	Gavage	5 weeks[6]	< 500	(Nagano et al., 1977)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M 3/NR	Gavage	5 weeks	1000[7]	(Nagano et al., 1979)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M3/NR	Gavage	5 weeks	< 500[8]	(Nagano et al., 1984)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
Butane-1,3-diol [02.132]	Rat; M 15/10	Diet	30 weeks	200000 ppm (10000 mg/kg/day)	(Miller and Dymsza, 1967)	Study aimed at elucidating the usability of butane-1,3-diol as synthetic energy source. It is of limited value for toxicological evaluation.
	Rat; M, F 3/60	Diet	2 years	100000 ppm (5000 mg/kg/day)	(Scala and Paynter, 1967)	Some details of results not reported (e.g. consumption, histopathological

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
						evaluation), limited value.
	Dog; M, F 3/8	Diet	2 years	30000 ppm (750 mg/kg/day)	(Scala and Paynter, 1967)	
	Dog; M, F 4/8	Diet	13 weeks	6000	(Reuzel et al., 1978)	Methods, results, discussion comprehensible. Valid study.
(4-Oxovaleric acid [08.023])	Rat: NR 2/3	Diet	16	1000[2]	(Tischer et al., 1942)	a)
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Rat; M, F 1/20 1/60	Diet	2 years	250[2]	(Bär and Griepentrog, 1967)	a)
Malonic acid [08.053]	Rat; M, F 3/140	Diet	2 years	10[9]	(Hogan and Rinehart, 1979)	
(Diethyl malonate [09.490])	Rat; M, F 2/20	Diet	13 weeks	< 500	(Posternak, 1964a)	a)
	Rat; M, F 1/20-32	Diet	90	40[2]	(Posternak et al., 1969)	a)
(Fumaric acid [08.025])	Rat 2/14 1/20	Diet[10]	2 years	1380[2]	(Levey et al., 1946)	a)
	Guinea pig; M, F 1/NR	Diet	1 year	400[2]	(Levey et al., 1946)	a)
	Rat; M, F Rat; M 4/12 3/12	Diet	2 years	1200	(Fitzhugh and Nelson, 1947)	a)
	Rabbit; NR 3/15	Diet[10]	150	2070[2]	(Packman et al., 1963)	a)
(Tartaric acid (d-, l-, dl-, meso-) [08.018])	Dog; NR 1/4	Oral	90-114	< 990	(Krop et al., 1945)	a)
	Rat; M, F 4/12	Diet	2 years	1200[2]	(Fitzhugh and Nelson, 1947)	a)
	Rabbit: NR 3/15	Diet[2]	150	2310[2]	(Packman et al., 1963)	a)
Glutaraldehyde [05.149]	Rat; M, F 4/10	Diet	7	1.0	(Union Carbide Corp., 1986)	
	Rat; M, F 3/NR	Drinking water	14	100 ppm (10 mg/kg/day)	(Union Carbide Corp., 1993)	
	Rat; NR 3/3	Drinking water	11 weeks	5000 ppm (500 mg/kg/day)	(Spencer et al., 1978)	
	Mouse; M, F 3/40	Drinking water	90	100 ppm (20 mg/kg/day)	(Bushy Run Research Center, 1989)	
	Rat; M, F 3/NR	Drinking water	13 weeks	50 ppm (5 – 7 mg/kg/day)	(Union Carbide Corp., 1986)	
	Dog;, M, F 3/8	Drinking water	13 weeks	50 ppm (3.2 mg/kg/day)	(Bushy Run Research Center, 1990)	
	Rat; M, F 3/200	Drinking water	2 years	50 ppm (4 mg/kg/day)	(Van Miller et al., 2002)	Large Granular Lymphocytic Leukemia in treated as well as control rats; no clear

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no:]	Species; Sex No./Group[1]	Route	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
						dose-resposne relationship. Otherwise no significant increase in neoplasia.
(Adipic acid [08.026])	Rat; M, F 4/20-39	Diet	2 years	~ 1500[11]	(Horn et al., 1957)	a)
Nonanedioic acid [08.103]	Rat; M, F 2/30	Diet	90 and 180	280	(Mingrone et al., 1983)	Details of methods not reported, study not performed according to appropiate guidelines. Study of limited value.
	Rabbit; M, F 2/20	Diet	90 and 180	400	(Mingrone et al., 1983)	
	Rat; F 1/10	Diet	3 month[12]	140	(Mingrone et al., 1983)	
	Rabbit; F 1/10	Diet	3 months[12]	200	(Mingrone et al., 1983)	
(Diethyl sebacate [09.475])	Rat; M, F 2/10	Diet	17-18 wks or 27-28 wks	1000[2]	(Hagan et al., 1967)	a)
	Rat; M 4/10	Diet	1 year	1250[2]	(Smith, 1953a)	a)
	Rat; M 5/16	Diet	2 years	6250[2]	(Smith, 1953a)	a)
(Triethyl citrate [09.512])	Rat; M, F 3/7	Diet	2 months	4000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 1/6	Gavage	2 months	< 285	(Finkelstein and Gold, 1959)	a)
(Tributyl acetylcitrate [09.511])	Rat; M, F 2/4	Diet	2 months	5000[2]	(Finkelstein and Gold, 1959)	a)
	Cat; NR 2/4	Gavage	2 months	< 5700	(Finkelstein and Gold, 1959)	a)
(Succinate, monosodium)	Rat; M,F 10/10	Drinking water	13 weeks	1250	(Maekawa et al., 1990) in (OECD, 2003)	
	Rat; M,F 50/50	Drinking water	2 years	2000	(Maekawa et al., 1990) in (OECD, 2003)	Monosodium succinate was given ad libitum in drinking water at levels of 0, 1, or 2% to F344 rats (50 males, 50 females). No toxic lesion specifically caused by long-term administration of monosodium succinate was detected.
(Succinate, disodium hexahydrate)	Rat; M,F 12 /12	Gavage 0, 100,300, 1000 mg/kg)	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	Males: 100 Females: 300	MHLW, Japan 2002 in (OECD, 2003)	combined repeated dose toxicity study with the reproduction/developmentaltoxicity screening test guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: males, 60 mg/kg; females. 180 mg/kg.

NR: Not reported

M = Male; F = Female.

a) Study summarised by JECFA at the 49th or 53rd meetings (JECFA, 1998a; JECFA, 2000c).

1 Number of groups represents the number of treatment groups investigated.Control groups are not reported.

2 This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.

3 Article published in Russian.Data point not verified.

4 Six animals per treatment group.The treatment groups for males were not the same as the females. Males were administered 2000 or 6000 ppm of the test substance, while the corresponding dose levels for the females were 1600 and 4800 ppm, respectively.

5 Compared to the control group absolute and relative thymus weights were significantly lower in males.These findings were not seen in females receiving up to 650 mg/kg/day.

6 Animals dosed 5 days a week for five weeks.

7 Changes in absolute or relative testis weights were not observed.

8 A decrease in red cell count was noted in the 500 mg/kg dose group and higher dose groups.

9 No treatment related effects were noted upon mortality, ophthalmology or body weights in the males. Microscopic evaluation noted that the transitional cell carcinomas were found in the urinary bladder. The findings were indicated to be dose related.

10 Administered as the sodium salt.

11 Rats fed a maximum dose of ca. 2500 mg/kg/day over a two-year period showed no gross or microscopic changes to their organs. There was no change in the incidence of tumours and mortality was unaffected. There was a slight reduction in body weight in animals dosed at ca. 1500 mg/kg/day and above.

12Animals were dosed for 19 gestational days prior to the three month exposure period that is reported.

13 The value of the study is limited by high mortality in all treatment and control groups.

Developmental and reproductive toxicity data are available for five candidate substances of the present Flavouring Group Evaluation from groups 9, 13 and 30 of the present Flavouring Group Evaluation and for two supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Furthermore, data are available for one structurally related substance. The supporting and structurally related substances are listed in brackets.

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
(Butyro-1,4-lactone [10.006])	Rat; F	Gavage	5/10	Developmental toxicity: Gestation days 6-15	500	(Kronevi et al., 1988)	
2-Butoxyethan-1-ol [02.242]	Mouse; M, F	Drinking water	5/16	FACB: (Task 1) 2 weeks	0.5 %[2] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	3/40	FACB: (Task 2) 14 weeks[3]	Reproductive: 0.5 %[4] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Drinking water	1/40	FACB: (Task 3) 14 weeks[3]	M: 1.0 % F: < 1.0 %[5] (2000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; M, F	Lactation/ Drinking water	1/40	FACB: (Task 4) 32 weeks	0.5 %[6] (1000 mg/kg/day)	(Gulati et al., 1985b; Heindel et al., 1990)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Rat; F	Gavage	3/45-47 3/52-59	Developmental toxicity: Gestation days 9 – 11 and 11 - 13	Maternal: 30 Fetal: 100	(Sleet et al., 1989)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	5/6	Developmental toxicity: Gestation days 8 - 14	Maternal: 1000 Fetal: 650	(Wier et al., 1987)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
	Mouse; F	Gavage	1/50	Developmental toxicity: Gestation days 6 – 13	Maternal: < 1180[7] Fetal: 1180[7]	(Hardin et al., 1987; Schuler et al., 1984; Smith, 1983)	FGE.10 refers to (EPA, 1999; EU-RAR, 2004a).
Butane-1,3-diol [02.132]	Rat; M, F	Diet	3/50	Five generations ~ 2 years	Reproduction: 5%[8] (5000 mg/kg/day) Teratogenicity: 5% (5000 mg/kg/day)	(Hess et al., 1981)	
	Rat; M, F	Gavage	3/10	Developmental toxicity: Gestation days 6 – 15	Maternal: 706; Fetal: 706	(Mankes et al., 1986)	
Glutaric acid [08.082]	Rat; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 1300 Fetal: 1300	(Bradford et al., 1984)	
	Rabbit; F	Gavage	3/NR	Developmental toxicity: NR	Maternal: 500 Fetal: 500	(Bradford et al., 1984)	
Glutaraldehyde [05.149]	Rat; M, F	Drinking water	3/56	Reproductive toxicity: 39 weeks[9]	Adult: 50 ppm (5.6 mg/kg/day) Fetal: 250 ppm (24.3 mg/kg/day) Reproductive: > 1000 ppm (84.5mg/kg/day)	(Neeper-Bradley and Ballantyne, 2000)	
	Rat; F	Drinking water	3/25	Developmental toxicity: Gestation days 6 – 16	Maternal: 50 ppm (5 mg/kg/day); Fetal: 750 ppm(68 mg/kg/day)[10]	(Hellwig, 1991a)	
	Rat; F	Gavage	3/21 – 26	Developmental toxicity: Gestation days 6 – 15	Maternal: 50; Fetal: 100	(Ema et al., 1992)	
	Mouse; F	Oral	3/NR	Developmental toxicity: Gestation days 7 – 12	Embryotoxicity: 30; Fetal: 30, Teratogenicity: 30	(Union Carbide Corp., 1986)	
	Rabbit; F	Gavage	3/15	Developmental toxicity: Gestation	Maternal: 15; Fetal: 15	(Hellwig, 1991b)	

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no:]	Species; Sex	Route	No. groups/ No. per group1	Duration (days)	NOAEL (mg/kg/day)	Reference	Comments
				days 7 – 19			
(Adipic acid [08.026])	Rat; F	Gavage	4/24-28	Developmental toxicity: Gestation days 6 – 15	288	(Morgareidge, 1973d)	
	Mouse; F	Gavage	4/20 – 21	Developmental toxicity: Gestation days 6 – 15	263	(Morgareidge, 1973d)	
	Rabbit; F	Gavage	4/10 – 14	Developmental toxicity: Gestation days 6 – 18	250	(Morgareidge, 1974a)	
Nonanedioic acid [08.103]	Rat; F	Diet	1/20	Developmental toxicity: Gestation days 0 - 19	140	(Mingrone et al., 1983)	
	Rabbit; F	Diet	1/30	Developmental toxicity: Gestation days 0 - 19	200	(Mingrone et al., 1983)	
(Succinate, disodium hexahydrate)	Rat; M,F	Gavage 0, 100,300, 1000 mg/kg)	4 per sex/ 12	Males: 52 days, starting at 14 days before mating. Females: Day 14 before mating until day 4 of lactation	M, F: 1000	MHLW, Japan 2002 in (OECD, 2003)	Combined repeated dose toxicity study with the reproduction/developmentaltoxicity screening test guideline [OECD TG 422]. Euqivalent NOAEL for sodium succinate: m, 600 mg/kg.

M = Male; F = Female.

NR = Not Reported.

FACB = Fertility Assessment by Continuous Breeding.

1 Number of groups represents the number of treatment groups investigated. Control groups are not reported.

2 Dose range-finding phase: Based on the results of this dose range-finding study the highest concentration investigated further was 2 % in the drinking water.

3 Mice were exposed to the test article for a seven day premating period, followed by a 14 week cohabitation/breeding period.

4 Continuous breeding phase: All breeding pairs in the 0.5 % treatment group were fertile (delivered at least one litter). The fertility of the 1.0 and 2.0 % treatment groups was significantly affected.

5 Crossover mating trial: Reproductive capacity of female mice is relatively more susceptible than males under the same exposure conditions.

6 Offspring reproductive performance phase: Reproductive performance was not affected, but the mean liver and kidney weights for females was significantly different from that of the control group when organ weight was adjusted for body weight.

7 1180 mg/kg/day was the only dose level tested. Compared to the control group the 1180 mg/kg/day decreased the number of viable litters; therefore increasing the number of failed pregnancies. There were no significant observations noted in the liveborn pups.

8 Dose related reproductive effects were noted after five successive matings of the F1A generation.

9 F0 and F1 animals dosed for a 10 week pre-breeding period and through mating, and gestation and lactation of offspring.

10 Glutaraldehyde was evidentially unpalatable, as water consumption was reduced in the mid- and high-dose groups; however, no signs of toxicity were observed at these dose groups.

In vitro mutagenicity/genotoxicity data are available for nine candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for 24 supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	Ames test	*S. typhimurium* TA98, TA100, TA1535	0.1 - 50 µmoles/plate (8.6 - 4305 µg/plate)	Negative[1]	(Loquet et al., 1981)	No control values are given for inactive compounds. Conclusion not comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA102	0.013 -1.3 mmol (11.2 - 1120 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(NTP, 1992e)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537,	5,000 or 2000 µg/plate	Negative[1]	(MacDonald, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	0 - 10000 µg/plate	Negative[1]	(Haworth et al., 1983)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	NR	Negative[1]	(Garner et al., 1981)	
	Ames test	*S. typhimurium* TA98,TA100, TA1535, TA1537, TA1538	4 - 2500 µg/plate	Negative[1]	(Trueman, 1981)	
	Ames test	*S. typhimurium* TA92, TA98, TA100, TA1535, TA1537, TA1538	0.2 - 2000 µg/plate	Negative[1]	(Brooks and Dean, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 µg/ml	Negative[1]	(Baker and Bonin, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Rowland and Severn, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	500 µg/plate	Negative[1]	(Simmon and Shephard, 1981)	
	Ames test	*S. typhimurium* TA98, TA100, TA1537	NR	Negative[1]	(Nagao and Takahashi, 1981)	
	Ames test	*S. typhimurium* TA98, TA100,	1000 mg	Negative[1]	(Ichinotsubo et al., 1981b)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 10000 µg/plate	Negative[3]	(Richold and Jones, 1981)	
	Reverse bacterial mutation assay	*E. coli* WP2 (p)	Up to 500 µg/plate (high dose studies) up to 100 µg/plate (low dose studies)	Negative[3]	(Venitt and Crofton-Sleigh, 1981)	
	Reverse bacterial mutation assay	*E. coli* SA500	NR	Lethal[4]	(Dambly et al., 1981)	Authors state "toxic, preventing adequate testing".
	Reverse mutation assay	*E. coli* WP2 *uvrA* pKM102	NR	Negative[1]	(Matsushima et al., 1981)	
	Forward mutation assay	*S. typhimurium* TM677	1000 µg/ml	Negative[3]	(Skopeck et al., 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA1535, TA1537	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Microtiter fluctuation test	*S. typhimurium* TA98, TA100	NR	Negative[3]	(Hubbard et al., 1981)	
(Butyro-1,4-lactone [10.006]) continued	Microtiter fluctuation test	*E. coli* WP2 *uvrA*	10 - 1000 µg/ml	Negative[3]	(Gatehouse, 1981)	
	Rec-assay	*Bacillus subtilis* H17, M45	20 µl (20000 µg)	Positive[1]	(Kada, 1981)	Reliable study, conclusion comprehensible.
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA, LexA*	NR	Negative[1]	(Green, 1981)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Differential killing test	*E. coli* WP2 *pol A*, WP2 *uvrA*, WP67 *uvrA*, WP67 *pol A*, CM871 *uvrA recA*, *LexA*	1000 µg/ml	Negative[2]	(Tweats, 1981)	
	Mitotic crossing-over	*S. cerevisiae*	1000 µg/ml	Negative[1]	(Kassinova et al., 1981)	
	Mitotic gene conversion	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	Cell growth inhibition	*S. cerevisiae* (JDI)	750 µg/ml	Negative[2]	(Sharp and Parry, 1981)	
	DNA polymerase I inhibition test	*E. coli* W3110 & P3478	10 µl (10000 µg)	Positive[2] Negative[3]	(Rosenkranz et al., 1981)	Reliable study, conclusion comprehensible.
	Forward mutation assay	*S. Pombe*	20 µg/ml[1]	Negative[3]	(Loprieno, 1981)	
	Unscheduled DNA synthesis	Human HeLa S3 cells	0.1 100 µg/ml	Negative[1]	(Martin and McDermid, 1981)	
	ADP-ribosyl transferase activity	Human FL cells	10^{-3} to 10^{-7} mol/L (0.0086 - 86 µg/ml)[3]	Negative	(Yingnian et al., 1990)	
	Clastogenic activity	Rat liver cell line RL1	250 µg/ml	Negative	(Dean, 1981)	
	Mammalian cell transformation	BHK-21 hamster kidney cells	250 µg/ml	Positive[1]	(Styles, 1981)	No specific genotoxicity endpoint.
	Degranulation assay	Rat	25 mg/ml (25000 µg/ml)	Positive	(Fey et al., 1981)	No genetic endpoint (displacement of polysomes from ER).
	Sister chromatid exchange	Chinese hamster ovary cells	494 - 4940 µg/ml 494 - 1480 µg/ml 3010 - 4940 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible.
	Chromosomal aberration	Chinese hamster ovary cells	400 - 2580 µg/ml 400 - 1500 µg/ml > 2580 µg/ml	Negative[2] Negative[3] Positive[3]	(NTP, 1992e)	Study in complinace with NTP laboratory health and safety requirements, conclusion comprehensible. Cells were selected for scoring on the basis of good morphology and completeness of karyotype.
Pentano-1,5-lactone [10.055]	Microbial assay	*E. coli* B/rWP2(*trp*$^-$), WP2(*trp*$^-$), WP2(*uvrA*$^-$)	1 - 3 mg/plate (1000-3000 µg/plate)	Negative[5]	(Kuroda et al., 1986)	Review, data cannot be validated.
(Hexano-1,5-lactone [10.010])	Ames test	*S. typhimurium* TA98, TA100	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Rec-assay	*B. subtilis*	NR	Negative[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Sister chromatid exchange	Hamster lung fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Hamster lung fibroblast cells	NR	Positive[2]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
	Chromosomal aberration	Human embryo fibroblast cells	NR	Negative[3]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Heptano-1,4-lactone [10.020])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	100,000 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Unscheduled DNA synthesis	Rat hepatocytes	3000 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
(Nonano-1,4-lactone [10.001])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	37500 µg/plate	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mammalian	Mouse lymphoma L5178y TK$^{+/-}$	1000 µg/ml	Negative[2]	(Heck et al., 1989)	Abstract only, study cannot be validated.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
			600 µg/ml	Positive[3]		
	Unscheduled DNA synthesis	Rat hepatocytes	500 µg	Negative[1]	(Heck et al., 1989)	Abstract only, study cannot be validated.
	Mutation assay	*E.coli* WP2 *uvrA*	0.2 - 1.6 mg/plate (200 - 1600 µg/plate)	Negative[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis* M45 & H17	20 µl/disk (20000 µg/disk)	Positive[4]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
(Undecano-1,4-lactone [10.002])	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537, TA2637	5 mg/plate (5000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test	*S. typhimurium* TA97, TA98, TA100,TA102	0.1 mg/disk (100 µg/disk)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[6]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis* H17 & M45	10 µl/plate (10000 µg/plate)	Positive[3] Negative[2]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast	0.5 mg/ml (500 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
(Undecano-1,5-lactone [10.011])	Rec-assay	*B. subtilis* H17 & M45	19 µg	Negative[1]	(Oda et al., 1979)	
	Rec-assay	*B. subtilis*	10 µl/plate (10000 µg/plate)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated, study cannot be validated.
(Pentadecano-1,15-lactone [10.004])	Ames test	*S. typhimurium* TA98, TA100, TA102	50 µmol (12 µg/ml)	Negative[1]	(Aeschbacher et al., 1989)	
(5-Methylfuran-2(3H)-one [10.012])	Ames test	*S. typhimurium* TA98, TA100	5 - 50 µg/plate	Negative[1]	(Turek et al., 1997)	
(Dodec-6-eno-1,4-lactone [10.009])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
	Rec-assay	*E. coli* WP2 *uvrA*	500 µg/plate	Negative[1]	(Watanabe and Morimoto, 1990)	
(3-Hydroxy-4,5-dimethylfuran-2(5H)-one [10.030])	Formation of 32P-labelled DNA fragment (test on isolated DNA).	*p53* tumour suppression gene	1mM (128 µg/ml)	Negative[7]	(Yamashita et al., 1998)	
1-Hydroxypropan-2-one [07.169]	Ames test	*S. typhimurium* TA100	20 - 400 µg/plate	Positive[1]	(Yamaguchi, 1982)	Effect dose-dependent, conclusion comprehensible.
	Ames test	*S. typhimurium* TA104	68 µmoles (5 µg/ml)	Positive[2]	(Marnett et al., 1985a)	Authors state that each compound was tested to its toxic limits, data for maximum non-toxic dose given only.
	Ames test	*S. typhimurium* TA100	500 µg/plate	Positive[1]	(Yamaguchi and Nakagawa, 1983)	Numerical value given was obtained from dose-response curves of five concentration levels.
	Ames test	*S. typhimurium* TA100	NR	Positive[2]	(Garst et al., 1983)	Appropriate controls (idomethan for volatile compounds, sterility of compounds and solvent). Test compound judged positive when dose-related doubling of revertants were found.
(Ethyl 3-hydroxybutyrate [09.522])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535	NR	Negative[4]	(Zeiger and Margolin, 2000)	
(Ethyl acetoacetate [09.402])	Ames test; preincubation protocol	*S. typhimurium* TA92, TA100, TA1535, TA1537 TA94 and TA98	25 mg/plate (25000 µg/plate)	Negative[1]	(Ishidate et al., 1984)	
	Ames test; preincubation protocol	*S. typhimurium* TA97, TA102	0.1 - 10 mg/plate (10 - 10000 µg/plate)	Negative[1]	(Fujita and Sasaki, 1987)	
	Rec-assay	*B. subtilis*, H17, M45	20 µg/disk	Negative[1]	(Oda et al., 1979)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Rec-assay	*B. subtilis*; H17, M45	20µl/disk (20000 µg/disk)	Positive	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*E. coli*; WP2 *uvrA*	200 - 1600µg/plate	Positive[8]	(Yoo, 1986)	Methods in Japanese, tables only in English. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20µl/ml (10 - 20 µg/ml)	Negative[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Rec-assay	*B. subtilis*; H17, M45	10 - 20µl/ml (10 - 20 µg/ml)	Positive[1]	(Kuroda et al., 1984a)	Abstract only translated. Study cannot be validated.
	Chromosomal aberration	Chinese hamster fibroblast cells	1 mg/ml (2000 µg/ml)	Negative[1]	(Ishidate et al., 1984)	
Methyl acetoacetate [09.634]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *E. coli* WP2 *uvrA*	1 - 5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	Modified Ames, reincubation. Reliable study, conclusion comprehensible.
2-Butoxyethan-1-ol [02.242]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10 - 5000 µg/plate	Negative[1]	(Okamoto and Riccio, 1985)	Study performed in compliance with US-FDA GLP standards. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 *uvrA*	9.8 - 156.3 µg/plate	Negative[1]	(Henrich and McMahon, 1988)	Test material: mixture of 2-butoxyethanol (2% w/v) with tricholorbenzene and anionic emulsifiers. Test compound produced no revertants vs solvent control..
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	100 - 10000 µg/plate	Negative[1]	(Zeiger et al., 1992)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537, TA1538	5000 - 20000 µg/plate	Negative[1]	(Sippel, 1977)	Negative as defined by less than 2-times of the spontaneous reversion rate. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA100 *E. coli* WP2uvrA	500 - 1000 µg/plate	Negative[1]	(Gollapudi et al., 1996)	Re-examination of EGBE to valdazte report by Hoflack et al (1995) on mutagenicity of the compound in a test with TA97a. reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, TA102	14 mg/plate (14000 µg/plate) conc. range: 0,8 - 115 micromol/plate, positive ab 19 micromol = 2,2mg/plate	Negative with TA98, TA100,TA102, positive with TA97a[1]	(Hoflack et al., 1995)	Positive with TA97a, but not reproduced in study specifically addressing this finding (Gollapudi et al., 1996).
	Mutagenicity Assay	Bacteriophage *T4D E. coli CR63* and *K12*	19.6 - 111.1 µl/ml	Negative[9]	(Kvelland, 1988)	Highly toxic at all concentrations tested, bacteriophage yield less than 1%.
	Forward mutation assay	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)[3]	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to currunt OECD guidelines.
	Forward mutation assay	Chinese hamster ovary cells V79	1 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study ((5 concentrations each test, 1% without S9 (non-toxic), 0,3 % with S9)), conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells AS52	0.38 - 7.6 mM (898 µg/ml)	Negative[1]	(Chiewchanwit and Au, 1995)	Non-cytotoxic concentration range. Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells	0.007 - 0.25 %	Negative[1]	(Slesinski and Weil, 1980)	Reliable study, conclusion comprehensible.
	Sister chromatid exchange	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Positive[2, 10]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to current OECD Guidelines.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Sister chromatid exchange	Human peripheral lymphocytes	3000 ppm	Positive[1]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
	Sister chromatid exchange	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells	5000 µg/ml	Negative[1]	(NTP, 2000b)	NTP-study within mutagenicity testing programme. Reliable study, conclusion comprehensible.
	Chromosomal aberrations	Chinese hamster ovary cells V79	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	Reliable report with details on purity of test compounds, methods and results. 50 % growth inhibition (at 24hours) approx. at 90 mM, but value cannot be precisely derived from the graphic presentation.
	Chromosomal aberrations	Human peripheral lymphocytes	3000 ppm	Negative[2]	(Villalobos-Pietrini et al., 1989)	Cited in review on 2-Butoxyethanol. Study cannot be evaluated.
2-Butoxyethan-1-ol [02.242] continued	Chromosomal aberrations	Human lymphocytes	16.92 mM (2000 µg/ml)	Negative[2]	(Elias et al., 1996)	No information on growth inhibition/ survival of treated human lymphocytes given.
	In vitro micronucleus test	V79 cells	16.92 mM (2000 µg/ml)	Positive[2]	(Elias et al., 1996)	It is noted that doses applied exceeded the maximum recommended doses according to currunt OECD Guidelines.
	Unscheduled DNA synthesis	Rat hepatocytes	0.1 100 x 10^{-3} %	positive[1, 11]	(Slesinski and Weil, 1980)	The interpretation of these findings is equivocal due to the methodology applied (liquid scintillation) and the absence of relation with dose.
	Embryo Transformation Assay	Syrian hamster embryo cells	NR	Negative[2]	(Elias et al., 1996)	No specific genotoxic endpoint.
	Embryo Transformation Assay	Syrian hamster embryo cells	500 - 1500 µg/ml	Positive[4]	(Brauninger, 1995)	No specific genotoxic endpoint.
(3,7-Dimethyloctane-1,7-diol [02.047])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Diethyl malonate [09.490])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537.	3 µmol/plate (480 µg/plate)	Negative[1]	(Florin et al., 1980)	
(Dimethyl succinate [09.445])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984)	
	Ames test	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538	10 mg/plate (10000 µg/plate)	Negative[1]	(Zeiger et al., 1992)	
(Fumaric acid [08.025])	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative[4]	(Rapson et al., 1980)	
	Ames test (preincubation)	*S. typhimurium* TA97, TA98, TA100, TA1535, TA1537	2000 µg/plate	Negative[1]	(Zeiger et al., 1988)	
	Ames test	*S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	10 mg/plate (10000 µg/plate)	Negative	(Ishidate et al., 1984)	

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
	Chromosomal aberrations	Chinese Hamster fibroblast cells	0.5 mg/ml (500 µg/ml)	Negative	(Ishidate et al., 1984)	
(l-Malic acid [08.017])	Ames test	*S. typhimurium* TA97, TA98, TA100, TA104	2000 µg/plate	Negative[1]	(Al-Ani and Al-Lami, 1988)	
Diethyl maleate [09.351]	Forward mutation assay	Mouse lymphocytes L5178Y TK+/-	2.250 – 9.750 x 10^{-3} mol/l (387 - 1679 µg/ml)	Positive[1]	(Wangenheim and Bolcsfoldi, 1988)	No S9 at 2.25 - 9.75 x 10^{-4} mol/L, doubling of the mutation rate at 6 x 10^{-4} mol/L and above, but growth reduction of 70 % or more. Study of insufficient value.
	Aneuploidy test	Chinese hamster lung cells V79	5.2 x 10^{-6} M 8.7 x 10^{-6} M	Negative[4] Positive[4]	(Önfelt, 1987)	Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	REC assay Ames	B subtilis M45 & H17 *S. typhimurium* TA98, TA100	NR	Negative[1]	(Sakagami et al., 1989)	Abstract, data cannot be validated.
Glutaraldehyde [05.149]	Ames test	*S. typhimurium* TA104	0.5 µmoles (50.06 µg/ml)	Positive[2]	(Marnett et al., 1985a)	TA104 tested to reassess mutagenic potency of 28 carbonyl compounds. Dose-dependent increase toxic limits of glutaraldehyde. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98	10 mg/plate (10000 µg/plate)	Equivocal[12] Positive[12]	(Haworth et al., 1983)	Part of ring study for re-assessment of 250 chemicals. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA100, TA102, TA104	25 - 300 µg/plate	Positive[1]	(Dillon et al., 1998)	Comparative analysis of TA102, TA104 and TA 102 for sensitivity to 13 aldehydes and 4 peroxides. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638, *E. coli* WP2/pKM101, WP2 *uvrA*	20 - 1000 µg/plate	Positive[3, *]	(Watanabe et al., 1998a)	*Cytotoxicity noted in doses as low as 250 µg/plate. Ring study (22 laboratories) for comparative analysis of TA102, TA2638, *E. coli* WP2/pKM101 and WP2 uvrA/pKM101. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, *E. coli* WP2/pKM101, WP2 *uvrA*	5 - 100 µg/plate	Positive[2]	(Wilcox et al., 1990)	Comparative analysis of TA102 and *E.coli* WP2 strains. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	1000 µg/plate	Positive[13]	(Müller et al., 1993)	Ring study (3 laboratories) to evaluate TA102. Reliable, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102, TA2638a	76 µg/plate	Positive[3, 14]	(Rydén et al., 2000)	Comparative analysis on the sensitivity of bacterial strains and the possibility of using TA2638a. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA102	25 µg/plate	Positive[1]	(Levin et al., 1982)	Test of TA102 for detection of oxidative mutagens. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, A102, TA104	0.1 - 60 µg/plate	Positive[1]	(Noblitt et al., 1992)	Abstract, data cannot be validated.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 *uvrA*	100 - 5000 µg/plate	Negative[1]	(Wagner, 1997)	Study in compliance with international (US-FDA, US-EPA, UK, Japan) GLP Guidelines. Negative result not discussed in

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
						view of positive results in other studies. Reliable study, conclusion comprehensible.
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	15.4 µg/plate[2, 15] 51.6 µg/plate[3]	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Ames test	*S. typhimurium* TA97a, TA98, TA100, A102, TA104	0.050 % in 100 µl/plate (100000 µg/plate)	Positive[14]	(Schweikl et al., 1994)	Study aimed at elucidating the mutagenic potency of 3 different dentin bonding agents, pure glutaraldehyde was tested as one of the ingredients of these materials. Conclusion comprehensible.
Glutaraldehyde [05.149] continued	Ames test	*S. typhimurium* TA100, TA98	20 µg/plate	Negative[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration Ames test less sensitive than REC-assay (see below).
	Ames test	*E. coli* WP2 *uvrA*	20 - 10000 µM (2 - 1001 µg/ml)	Negative[2]	(Hemminki et al., 1980)	Study aimed at comparison of alkylation rate with mutagenicity of directly acting chemicals, glutaraldehyde served as reference compound.
	Rec-assay	*B. subtilis*, M-45 (*Rec*$^-$), H-17 (*Rec*$^+$)	300 µg/ml	Positive[1]	(Sakagami et al., 1988)	Dose-dependent DNA-damage. At minimum inhibitory concentration REC-assay more sensitive than Ames test (see above).
	L-arabinose resistance forward mutation test	*S. typhimurium:* BA9, BA13	62 - 250 nmoles/ml (6.2 - 25 µg/ml)	Negative[15] Positive[15]	(Ruiz-Rubio et al., 1985)	
	Forward mutation assay	Mouse lymphocytes: L5178Y TK+/-	8 µg/ml	Positive[2]	(McGregor et al., 1988b)	Reliable study, conclusion comprehensible.
	Forward mutation assay	Chinese hamster ovary cells	40.8µM (4.08 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	2.5 µM (.25 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Sister chromatid exchange	Chinese hamster ovary cells	0.5 – 16 µg/ml	Negative/positive[2] Positive[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol.11-16 µg/ml, with S9 positive (at least with one dose) results in both laboratories. 0,36-16 µg/ml, without S9 results not consistent.
	Chromosomal aberrations	Chinese hamster ovary cells	0.5 - 30 µg/ml	Negative/positive[2] Negative[3]	(Galloway et al., 1985)	Study performed in 2 laboratories aimed to develop sensitive test protocol. 1-16 µg/ml, with S9 negative results in both laboratories: 0,3-30 µg/ml, without S9 results not consistent.
	Alkaline elution assay	Human TK6 lymphoblasts	25 µM (0.25 µg/ml)[2]	Positive[2]	(St. Clair et al., 1991)	Linear increase in DNA cross linking between 1-25 µM. At 20 µM 10 % survival only.
	TK6 mutation assay	Human TK6 lymphoblasts	20 µM (2 µg/ml)	Positive	(St. Clair et al., 1991)	Majority of trifluorothymidine resistant colonies displayed normal growth, slow-growing colonies small contribution to overall mutant fraction.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no:]	E ndpoint	Test Object	Concentration / Dose	Result	Reference	Comments
Glutaraldehyde [05.149] continued	Unscheduled DNA synthesis	Primary rat hepatocytes	51 µM (5.1 µg/ml)	Negative[1]	(Slesinski et al., 1983)	Lack of mutagenic activity considered to be due to reaction of glutaraldehyde with proteins in cell membrane, cytosol.
	Unscheduled DNA synthesis	Rat hepatocytes	100 µM (10 µg/ml)	Positive[2]	(St. Clair et al., 1991)	Significant increase over controls at 100 µM, this concentration tolerated without morphological signs of toxicity.
(Adipic acid [08.026])	Ames test	*E. coli* WP2 *uvrA*	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98, *E. coli* WP2 *uvrA*	10 mg/plate (10000 µg/plate)	Negative[1]	(Prival et al., 1991)	
	Ames test (preincubation method)	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	5000 µg/plate	Negative[1]	(Shimizu et al., 1985)	
(Dibutyl sebacate [09.474])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Ethylene brassylate [09.533])	Ames test	*S. typhimurium* TA1535, TA100, TA1537, TA1538, TA98	3.6 mg/plate (3600 µg/plate)	Negative[1]	(Wild et al., 1983)	
(Prop-1-ene-1,2,3-tricarboxylic acid [08.033])	Ames test	*S. typhimurium* TA100, TA1535, TA1537, TA98	20000 µg/plate	Negative[1]	(Andersen and Jensen, 1984a)	
5,6-Dimethyl-tetrahydro-pyran-2-one [10.168]	Ames test	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	5000 microgram/plate	Negative[1]	(Uhde, 2004a)	Test performed both in the incorporation and preincubation assays.
Succinic acid, disodium salt [08.113]	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537.	5000 microgram/plate	Negative3	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Ames test	*S.typhimurium* TA97, TA 102	10000 microgram /plate	Negative[1]	(Fujita et al., 1994) in (OECD, 2003)	GLP-study according to OECD TG 471.
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	15000 microgram/ml	Equivoval[2]	(Ishidate et al., 1984) in (OECD, 2003)	GLP-study according to OECD TG 473.
(Disodium succinate hexahydrate)	Ames test	*S.typhimurium* TA97, TA94, TA98, TA100, TA1535, and TA1537.	5000 microgram/plate	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	
	Chromosomal aberrations (polyploidy)	Chinese hamster lung cells	5000 microgram/ml	Negative[1]	MHLW, Japan 2002 in (OECD, 2003)	

NR: Not reported

1 With and without S-9 metabolic activation.

2 Without S-9 metabolic activation.

3 With S-9 metabolic activation.

4 Presence or absence of metabolic activation not specified.

5 Anti-mutagenic effects study.

6 Presence or absence of metabolic activation not specified.

7 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one did not form DNA adducts, but 2,5-DMHF does. Study addresses mechanism of chemical reaction of 2,5-dimethyl-4-hydroxy-3(2H)-furanone with DNA.

8 The concentrations used were 10-fold higher than that of spontaneous revertants.

9 The test substance had a severe toxic effect on phage yield.

10 Weak positive results were detected.

11 The test substance induced statistically significant levels of unscheduled DNA synthesis in two of the six dose levels tested. Therefore, the test substance is considered a weak mutagen.

12 This test compared the results at two different laboratories. Results were equivocal at Case Western Reserve University, while they were positive at Microbiological Associates.

13 Article presents the results from three different laboratories. Results were positive in both water and ethanol; however, it was concluded that TA102 is not sufficiently matured to be employed routinely.

14 Maximum non-toxic dose.

15 Results were negative in BA9, not BA13.

In vivo mutagenicity/genotoxicity data are available for six candidate substances of the present Flavouring Group Evaluation from chemical groups 9, 13 and 30 of the present Flavouring Group Evaluation and for eight supporting substance evaluated by JECFA at the 49th and 53rd meetings (JECFA, 1998a; JECFA, 2000c). Supporting substances are listed in brackets.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Butyro-1,4-lactone [10.006])	*In vivo* Bone- marrow micronucleus assay	B6C3F1 mice	Single dose *via* intraperitoneal injection	80 % of LD_{50}	Negative	(Salamone et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo Bone- marrow micronucleus assay	CD-1 mice		0.11- 0.44 ml/kg (110 - 440 mg/kg)	Negative	(Tsuchimoto and Matter, 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo micronucleus assay	Mice (B6C3F1/BR hybrid)		80 % of LD_{50}	Negative	(Katz et al., 1981)	Limited relevance because PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo sperm abnormality	Mice (CBA X Balb/c)F1	Daily exposure for five days *via* intraperitoneal injection	0.1-1.0 mg/kg bw/day	Negative	(Topham, 1980)	Sperm head abnormality test does not make use of a genetic endpoint.
	In vivo sex- linked recessive test	*D. melanogaster*	*A: via* diet B: injection	A: 20000 or 28000 ppm B. 15.000 ppm	Negative	(Foureman et al., 1994)	Study in compliance with OECD 477.
(Hexano-1,5-lactone [10.010])	Chromosomal aberration *in vivo*	Rat bone-marrow cell		NR	Negative[1]	(Kawachi et al., 1980b)	Summary of results on 186 compounds. No details on methods, concentrations and data given, results cannot be validated.
(Undecano-1,4-lactone [10.002])	*In vivo* mouse micronucleus test	2-6 ddY male mice	*Via* intraperitoneal injection	250-2000 mg/kg	Negative	(Hayashi et al., 1988)	Single application, only one sampling time. Not in compliance with current OECD 474.
2-Butoxyethan-1-ol [02.242]	*In vivo* mouse micronucleus test	Mouse bone marrow	Single dose *via* intraperitoneal injection	1000 mg/kg	Negative	(Elias et al., 1996)	Reliable report, decreased PCE/NCE ratio demonstrates bioavailability of compound at target compartment. Conclusion comprehensible.
2-Butoxyethan-1-ol [02.242] continued	*In vivo* mouse micronucleus test	Mouse bone marrow	3 doses *via* intraperitoneal injection	450 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo micronucleus test	Rat bone marrow	3 doses *via* intraperitoneal injection	550 mg/kg	Negative	(NTP, 2000b)	NTP-study within mutagenicity testing program. Reliable study, conclusion comprehensible.
	In vivo DNA adducts	Rat brain, kidney, liver, spleen and testes	Single dose *via* oral route	120 mg/kg	Negative	(Keith et al., 1996a)	The method (based on ^{32}P-postlabelling) is aimed at detecting hydrophobic DNA

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							adducts resulting from CytP450 induction, not from binding of 2-butoxyethan-1-ol to DNA.
	In vivo DNA methylation	Rat brain, kidney, liver, spleen and testes,	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo DNA adducts	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Detection of hydrophobic DNA adducts such as modified nucleotides with aliphatic side chains.
	In vivo DNA methylation	Mouse	*Via* oral route	NR	Negative	(Keith et al., 1996a)	Supplementary information not directly relevant for genotoxicity assessment.
	In vivo tumour formation	Mouse	Daily dose for two weeks *via* oral route	120 mg/kg/day	Inconclusive	(Keith et al., 1996a)	No difference in tumor incident observed. However no conclusion on the oncogenic potential of 2-butoxyethan-1-ol can be drawn because of the limitations of the experimental protocol (treatment, sample size, duration of the study, reporting, etc.).
Butane-1,3-diol [02.132]	*In vivo* cytogenetic assay	Rat femur bone marrow	*Via* diet[2]	5, 10, 24 %	Negative	(Hess et al., 1981)	F1A, F2A, F3A generations in a multigeneration reproductive toxicity study. PCE/NCE ratio was not reported, thus it is not clear if the test substance reached the bone marrow.
	In vivo dominant lethal assay	Rat	Animals exposed for eight weeks *via* diet	5, 10, 24 %	Negative	(Hess et al., 1981)	F1B generation in a multigeneration reproductive toxicity study
(3,7-Dimethyloctane-1,7-diol [02.047])	*In vivo* micronucleus test	Mouse		516, 860, 1204 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		10 mM (1743 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
(3,7-Dimethyl-7-hydroxyoctanal [05.012])	*In vivo* Basc test	*D. melanogaster*		37 mM (6374 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		345, 603, 861 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							clear whether the substance had reached the bone marrow.
(1,1-Dimethoxy-3,7-dimethyloctan-7-ol [06.011])	*In vivo* Basc test	*D. melanogaster*		25 mM (5459 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.
	In vivo micronucleus test	Mouse		327, 545, 763 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
Malonic acid [08.053]	*In vivo* mutagenicity assay	Rat hepatocytes	400 mg/kg/day exposure for 6 weeks *via* diet	4000 ppm	Negative	(Ito et al., 1988)	GST-P foci assay following diethyl nitrosamine exposure. Reliable study, conclusion comprehensible.
Glutaric acid [08.082]	*In vivo* bone marrow chromosomal aberrations	Rat bone marrow	Single dose *via* oral gavage	Males: 2750 mg/kg Females: 1375mg/kg	Negative	(San Sebastian, 1989a)	Reliable study, e.g. cells with gaps excluded. Selected copy of report without data tables.
Glutaraldehyde [05.149]	*In vivo* chromosomal aberration	Rat bone marrow	Single dose *via* oral gavage	Males: 120 mg/kg/bw Females: 80 mg/kg/bw	Negative	(Vergnes and Morabit, 1993a)	Study in compliance with international (FDA, TSCA, OECD) GLP guidelines. Selected copy of report (12 of 100 pages) available.
	In vivo chromosomal aberration	Rat bone marrow	A single dose or daily for five days *via* oral gavage	Single dose: 0.55 ml/kg (males), 0,4 ml/kg (females) of a 6, 12 or 36% solution. Repeated dose: 0,55ml/kg (males) of a 5% solution	Negative	(Putman, 1987)	Time points of investigation: single dose: 8,12 hou rs. Repeated dose: 12hrs. Well conducted study, conclusion comprehensible. Selected copy of report available.
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* oral gavage	250 mg/kg	Negative	(Vergnes and Morabit, 1993b)	Selected pages of report available (29 of 88 pages).
	In vivo mouse blood micronucleus test	Mouse	Single dose *via* intraperitoneal injection	4, 8, 15 mg/kg/bw	Positive	(Noblitt et al., 1993)	Abstract, study cannot be validated.
	In vivo unscheduled DNA synthesis	Rat	Single dose *via* oral gavage	30, 150, 600 mg/kg	Negative	(Mirsalis et al., 1989)	Reliable part of *In vivo* tumour formation study, conclusion comprehensible.
	In vivo SLRL test	*D. melanogaster*	Three day exposure *via* diet	3500 ppm	Negative	(Zimmering et al., 1989)	Study in compliance with OECD477.
	In vivo SLRL test	*D. melanogaster*	Single dose *via* intraperitoneal injection three day exposure *via* diet	Injection: 4000 ppm Diet: 10,000 ppm	Negative	(Yoon et al., 1985)	Study in compliance with OECD477.
(Adipic acid [08.026])	*In vivo* chromosomal nondisjunction	*D. melanogaster*		4000 ppm	Negative	(Ramel and Magnusson, 1979)	
Diethyl adipate [09.348]	*In vivo* dominant lethal assay	Mouse	(Single 1460 mg/kg dose *via* intraperitoneal injection)	1.46 ml/kg	Negative	(Singh et al., 1975)	Reliable study, conclusion comprehensible.

Table IV.5: Genotoxicity Studies (*In Vivo*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Dibutyl sebacate [09.474])	*In vivo* micronucleus test	Mouse		943, 1886, 2829 mg/kg	Negative	(Wild et al., 1983)	Limited quality since only a single sampling time (30 hours after treatment) was used and PCE/NCE ratio was not reported. Therefore it is not clear whether the substance had reached the bone marrow.
	In vivo Basc test	*D. melanogaster*		19 mM (4642 µg/ml)	Negative	(Wild et al., 1983)	A single dose was tested in one experiment. Method not described in detail.

NR: Not reported.

1 Presence or absence of metabolic activation not specified.

2 Length of exposure not specified in report. Cytogenetic assay conducted on F1A, F2A and F3A generations of a multiple generation study.

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ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADI	Acceptable Daily Intake
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CNS	Central Nervous System
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DRF	Dose Range Finder
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EPA	Environmental Protection Agency
ER	**E**ndoplasmic Reticulum
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
I.V.	Intravenous
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50%; Median lethal dose
LOAEL	Lowest Observed Adverse Effect Level
MFD	Median Fatal Dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate

No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
RfD	Reference dose
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation